Acta

OTO-LARYNGOLOGICA

VOL 89 MAY–JUNE 1980 No 1–6

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Conferences and Meetings

1980 Jan. 14–18 XVth Annual Otologic Surgery Course will be held at the Ear Research Institute in Los Angeles, USA. Further information. F H. Linthicum, Jr M.D., Ear Research Institute, 256 South Lake Street, Los Angeles, CA 90057 USA.

1980, Febr 4–March 15 Otolaryngology Pediatric Course to be held in Barcelona, Spain. Further information: Catedra de O.R.L., Facultad de Medicina, Calle Casanova 143 Barcelona-36, Spain.

1980 March, April, June, October· Two-weeks Temporal Bone Surgical Dissection Courses will be held at the Ear Research Institute, Los Angeles, USA. Further information. Antonio De La Cruz, M.D., Director Temporal Bone Surgical Dissection *Course, Ear Research Institute, 256 South Lake Street, Los Angeles, CA 90057* USA.

1980 March 2–8 Departments of Otolaryngology of University of Toronto and University of Pittsburgh announce Winter Meeting 1980—Mont Tremblant Lodge. Addr Dr William Crysdale, Suite 6118, 555 University Avenue, Toronto, Canada M5G 1X8

1980, March 3–7 Six Shambaugh International Workshop on Otomicrosurgery and Third Shea Fluctuant Hearing Loss Symposium. For further information Write Mr Herbert Carroll, National Hearing Association, 1010 Jorie Boulevard, Suite 308 Oak Brook, IL 60521 USA.

1980, April 8–12. First World Biomaterials Congress will be held in Baden near Vienna. Information World Biomaterials Congress Secretariat, Mrs E. Maurer Medical Academy of Vienna, Alser Strasse 4 A 1090 Vienna, Austria.

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1980 April 20–26. To celebrate the Centenary of the "Revue de Laryngologie, Otologie, Rhinologie" and of the "Ecole d'Oto-Rhino-Laryngologie" scientific and social manifestations are planned in Bordeaux, I. International Postgraduate Course and II. International Symposium. Further information for Secrétariat du Centenaire, 114 avenue d'Arès, 33 000 Bordeaux, France.

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THE VASCULAR ANATOMY OF THE RAT COCHLEA

C. Hornstrand A Axelsson and D Vertes

From the Department of Otolaryngology University of Göteborg Goteborg Sweden

(Received March 30 1979)

Abstract The vascular pattern of the rat cochlea was demonstrated by the aid of Berlin Blue (Prussian Blue) injection. The vascular anatomy is similar to other mammals. The infrequent occurrence of the vessel of the basilar membrane under the organ of Corti is interesting in respect to its presumed importance for the oxygen supply to the hair cells. In the place where the vessel of the basilar membrane usually lies when present, large uninjected vessels are often seen throughout the cochlea. In the spiral wall, radiating arterioles supply and collecting venules drain all capillary areas. The vascular pattern of the external wall is well maintained at the basal end but more sparse in apical parts of the cochlea as in other mammals. The rat cochlea is somewhat more difficult to manipulate than the cochlea in other mammals due to its small size, but the dissection of different parts of the cochlea was achieved without major problems

The rat is a very commonly used animal in research Both normotensive and hypertensive rats have been used as models in cardiovascular studies (Hallbäck & Folkow 1974 Ingvar 1974 Borg, 1976) Devine & Simpson (1967) used the rat vascular system to study the structure of sympathetic neuromuscular contacts The autonomic nerve supply to the inner ear vasculature of the rat has also been investigated (Spoendlin & Lichtensteiger 1966) Further small blood vessels of various organs and tissues in the rat have been a subject of research by means of electronmicroscopy (Rhodin 1977) The fact that the rat is resistant to middle ear infection has an accessible inner ear and can be trained by behavioural techniques has made it a very popular subject for electrophysiological (Møller 1970) as well as psycho-physical measurements.

The anatomy of the cochlear vasculature of the rat has been described in the early literature (Asai 1908 Nabeya, 1923) Recently Wicke (1978) has studied the spiral ganglion blood supply in the rat cochlea Hodde et al (1977) and Miodonski et al (1978) have also presented excellent treatises on the rat vasculature While the overall vascular pattern was quite well demonstrated by their microcorrosion cast technique detailed information on particular vessels of the spiral lamina was not given. The aim of the present investigation was to complete the picture of the cochlear vascular pattern in the rat.

MATERIAL AND METHODS

Eight rats each weighing approximately 250 g. were used in these experiments The demonstration of the cochlear blood vessels was achieved by a previously described method (Axelsson 1968 1971 1972 1973 Axelsson & Lind 1973) which in summary contains the following preparative steps Anesthesia with intraperitoneal administration of urethane perfusion of the vascular system with Ringer's solution under hydrostatic pressure injection of a contrast, Berlin Blue (Prussian Blue) solution under the same hydrostatic pressure fixation of the cochlea, decalcification washing dehydration, storage in glycerine microdissection of the cochlea using the stereo-microscope photographic registration of the microscopic findings using the light and phase contrast-microscope (Leitz Orthoplan)

Almqvist & Wiksell, Uppsala 1980

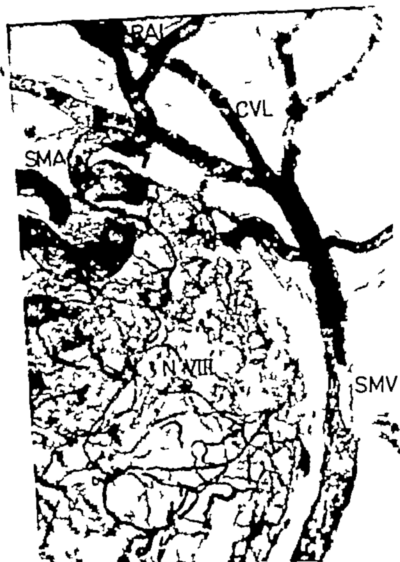


Fig 2 Rat cochlea, transverse section. Modiolus, turn 1 *SMA*, spiral modiolar artery branching into two *RAL*, radiating arterioles Branches of *CVL*, collecting venules, emptying into *SMV* spiral modiolar vein, *N VIII* acoustic nerve.

pig but run a more serpentine winding course The rat cochlea is drained by the spiral modiolar vein (SMV) which also runs a spiral course around the modiolus from the apex to the base (Fig.) SMV is situated in the basal central angle of the scala tympani below the spiral ganglion. SMV receives many collecting venules (CVL) from the external wall the spiral lamina and limbus vessels and the capillary areas in the modiolus In one animal a vessel was found running from the modiolus through the scala tympani to the external wall This vessel is previously termed the "suspension vein" when found in the guinea pig Similar to the guinea pig the "suspension vein" could be demonstrated in the hook" region near the round window

Spiral lamina

The following vessels can be identified in the spiral lamina. Radiating arterioles RAL, collecting venules CVL, vessel of the tympanic lip VSTL, limbus vessels LVS vessel of the basilar membrane VSBM.

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g 4 Rat cochlea, spiral lamina, turn 1.5 *VSTL*, vessel f the tympanic lip which often forms two spiral vascular orders at the tympanic lip. A loop formation of vascular connections between these two border vessels is often seen. *OHC* outer hair cells.

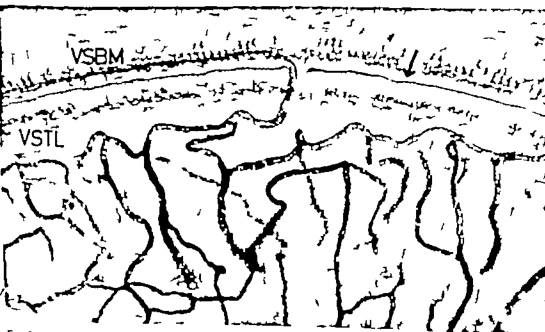


Fig 5 Rat cochlea, spiral lamina, turn 1. *VSTL*, vessel of the tympanic lip. *VSBM* vessel of the basilar membrane. Channel without contrast (arrow)

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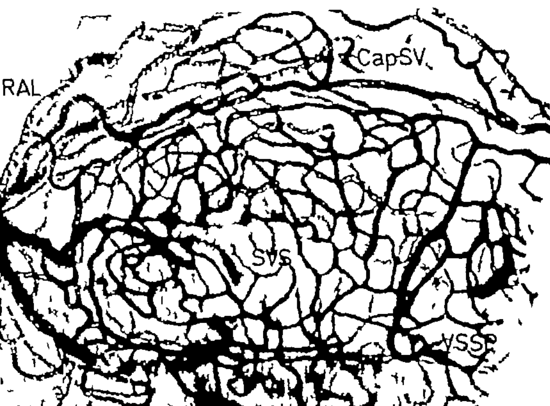


Fig. 9. Rat cochlea, extreme basal end. *RAL*, radiating arterioles; *CapSV*, capillaries of the scala vestibuli; *SVS*, stria vascularis; *VSSP*, vessel of the spiral prominence.

dially running CVL and these in turn often run in a fairly short distance radially before they empty in a large, spirally running collecting venule. Some of the CVL ST draining the stria vascularis and the arterio-venous anastomoses run directly to such a large collecting venule. In this way the appearance of the scala tympani is much less vascularized, particularly in its basal portions, than in other mammals.

Apex

Apical parts of the rat cochlea are characterized by the same simplification of the vascular pattern that was found in other mammals (Fig. 8). Despite the general simplification most of the regularly occurring vessels can be demonstrated here.

Basal end

The vascular pattern of the basal end is very similar to that in other mammals investigated. The basal end is supplied by the vestibulo-cochlear artery and drained by the vein of the round window (Fig. 1). In the region between the round window and the oval window, the "hook" region, the radiating arterioles coming from the vestibulo-cochlear artery enter the stria vascularis with a more oblique course. Other branches from the vestibulo-cochlear artery run in a retrograde apical direction after turning around the extreme basal end of the cochlea. These retrograde obliquely-running radiating arterioles also supply the capillary areas in the external wall. In the region where the radiating arterioles from both directions merge between the windows, an anastomosing

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5 min following HRP injection were required before Karnovsky (1967) found capillary basal lamina densely stained Although transport of HRP in muscle capillaries was primarily by means of pinocytosis intercellular cleft transport also occurred

In contrast with other permeability studies, Isako & Hilding (1971) have reported that HRP is transported across strial capillaries by a route between endothelial cells and that micropinocytotic vesicles are absent within strial endothelia of both Ames Waltzer mice and normal Swiss strain mice

Yamamoto & Nakai (1964) studied strial capillary transport in the Guinea pig using iron dextran At no time point after injection (10 min to 7? h) was the tracer observed in capillary basal lamina, although membrane bound accumulations were frequently found within endothelia. While these authors determined the size of iron dextran to be 100 Å in diameter others have shown that the diameter of this tracer ranges from 20–70 Å (Muir & Goldberg, 1961 Richter 1959) Molecules this size should be transported across strial endothelia if indeed the HRP results are sound In fact if one considers that the transport rate of HRP (mol diam 55 Å) is more rapid through strial than muscle capillaries and that transport in both cases primarily involves pinocytosis then it is difficult to account for the iron dextran results in light of the successful transport of molecules as large as ferritin (mol. diam 110 Å) across muscle endothelia (Bruns & Palade 1968) Clearly the literature on strial capillary permeability is in conflict.

It should be noted that the use of HRP as a tracer to study strial capillary permeability has certain drawbacks For example the enzyme HRP was noted by Duvall et al (1971) to cause strial capillary alterations consisting of intraluminal protrusions of endothelial cell membranes and pores within capillary walls. Cell protrusions have been known to occur in injured cells (Trump & Arstila, 1974) The artifactual production of pores in strial capillaries could have resulted in the release of HRP to the basal lamina. Such changes do not occur in adjacent spiral ligament vessels where no HRP exists Whether strial capillary alteration is a manifestation of HRP toxicity to select portions of the inner ear is not clear however it has been found that perilymphatic perfusion of HRP in vivo is acutely ototoxic (Ross et al. 1977)

Other problems of HRP use include (1) the enzyme must retain its catalytic activity until and throughout incubation (2) quantitative analysis of transported tracer substance cannot be performed since a) the HRP itself is not directly visualized and b) the quantity and quality of the reaction product is dependent upon the quality of both the enzyme preparation and incubation medium the penetration of the medium into the tissue and the duration of the incubation (3) histamine which affects vessel permeability and strial capillary blood flow is released in some species in response to HRP injection (4) an endogenous peroxidase reaction exists which may interfere with the interpretation of experimental findings

These problems are especially troublesome when evaluating capillary permeability changes in experimentally induced pathologies For example Duvall et al (1974) used the tracer substance HRP to evaluate strial capillary permeability after intense noise exposure They suggested that some of their unusual experimental results may have been due to the degradation of the protein HRP by lysosomal enzymes. Of course *if HRP is inactivated by* lysosomal hydrolases then the electron dense HRP reaction product which indicates the presence of HRP will not be produced Therefore the transport of the tracer cannot be determined.

Certainly then HRP and similar enzymatic tracers pose serious problems when used as a means to gage strial capillary transport during pathologic as well as normal conditions A reliable method of evaluating the function of strial capillaries without the limitations imposed by the use of an enzymatic tracer would be beneficial.

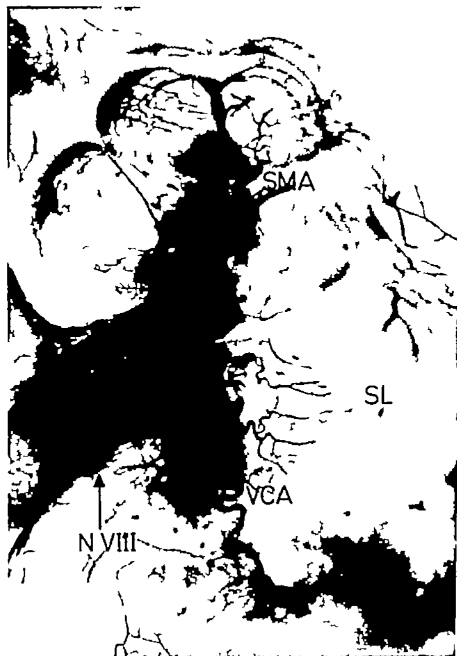
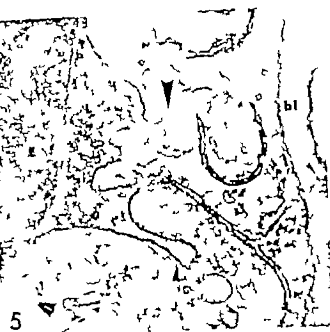


Fig 1 Rat cochlea, apico-basal paramodiolar section *SMA* spiral modiolar artery *VCA* vestibulo-cochlear artery *SL* spiral lamina, N VIII acoustic nerve

RESULTS

A more or less good contrast injection of the cochlear vascular system was achieved in all animals As in other mammals some parts of the capillary areas are often less well injected than others in the immediate vicinity This appears to be more the result of technical defects than due to pathological conditions in the vascular system

The rat cochlea has three turns In principle the blood supply to the rat cochlea appears to be very similar to that of the guinea pig The arterial supply is maintained by a large artery the spiral modiolar artery (SMA) running spirally around the modiolus from the base to the apex (Figs 1 and 2) SMA is situated between the acoustic nerve and the spiral ganglion at the level of the scala vestibuli Radiating arterioles (RAL) leave the spiral modiolar artery to supply the capillary areas in the modiolus in the scala vestibuli and the scala media and in the spiral lamina and limbus (Fig. 3) RAL do not form spring coils as previously described in the guinea



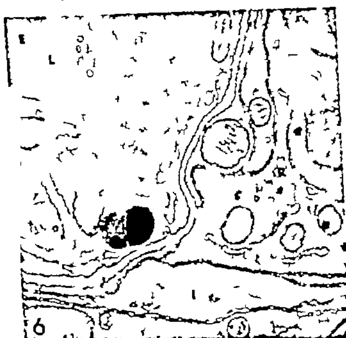


Fig 5 Large arrow indicates open-mouthed vacuole containing large amount of ferritin on luminal front. Medium arrow indicates luminal tubular invagination containing ferritin. Double arrow indicates fusion of two labeled pinocytotic vesicles. Ferritin molecule can be seen in basal lamina (small arrow) ½ h post injection. Glutaraldehyde and osmium fixed. Unstained. Mid portion of cochlear duct. ×90 025

Fig 6 Control animal (uninjected) showing light cell (*R*) with abundant supply of endogenous ferritin-like granules. Osmium fixed. Unstained ×39 600.

vesicles were observed within endothelial cells (Figs. 5, 7, 8 and 9). Labeling of any pinocytotic vesicles was limited to no more than a few molecules of ferritin. In any section the number of such labeled vesicles per endothelial cell varied from none to a few, with no discernible relation to time after injection or area of cochlear duct. At times the number of unlabeled vesicles was great. Although endothelial vesicles opening to the tissue front were

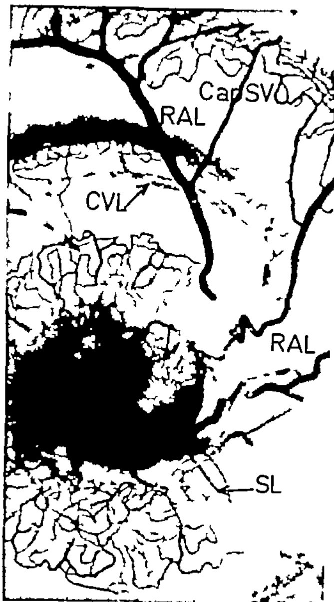


Fig 3 Rat cochlea, transverse section turn apical view *RAL* radiating arterioles supplying the external wall *CapS* capillaries of the scala vestibuli *CVL* collecting venules draining the external wall *SL*, spiral lamina.

Radiating arterioles and collecting venules (RAL–CVL) RAL supplying the capillary vessels are branches from the spiral modiolar artery They run centrifugally over the spiral lamina (Fig 3) Each RAL supplies a rather small segment When RAL reach the vessel of the tympanic lip or the vessel of the basilar membrane they turn off squarely in T junctions or build up arcades CVL drain the vessel of the basilar membrane the vessel of the tympanic lip and the limbus vessels and run centripetally over the spiral lamina to empty in the spiral modiolar vein

The vessel of the tympanic lip (VSTL) VSTL is a well-supplied and well-drained vessel which is found throughout the cochlea and is typically well injected by contrast It forms one or often two spiral marginal vascular borders in the tympanic lip (Fig 4) There is often a system of vascular loops connecting these two inner spiral vessels in addition to the more central loops in the spiral lamina In this way the vessels appear to form a capillary net in the spiral lamina which end peripherally in one or two vascular margins of VSTL.

The limbus vessels (LVS) LVS make up a capillary network peripherally in the spiral limbus The loops are sparse throughout the entire cochlea Different branches from the same radiating arterioles and collecting venules are provided for the VSTL and VSBM and the LVS and there are no direct connections between those spiral vessels and the LVS The blood supply to the spiral limbus appears poor in the rat in comparison with other mammals

The vessel of the basilar membrane (VSBM) VSBM is not very often seen However large uninjected channels resembling the previously termed "avascular channels" are almost always seen especially in the basal turn under the tunnel of Corti where VSBM usually lies when present (Fig 5) In cases where the vessel was present it was a single injected vessel without branches The non-contrast injected channels themselves appear to be long—extending over a whole turn—with very few supplying radiating arterioles or draining collecting venules At the so-called T junction where supplying and draining radiating arterioles and collecting venules join VSBM one can sometimes see an injected VSBM within a perivascular space running spirally in one direction while a channel without contrast continues spirally in the other direction (Fig 5) Nothing was ever seen at the T junction to inhibit the injection of Berlin Blue

The vestibular and tectorial membranes are


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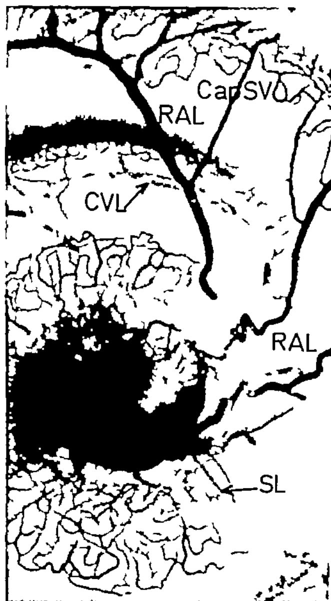


Fig 3 Rat cochlea, transverse section turn 2 apical view *RAL* radiating arterioles supplying the external wall *CapSv* capillaries of the scala vestibuli *CVL* collecting venules draining the external wall *SL* spiral lamina

Radiating arterioles and collecting venules (RAL–CVL) RAL supplying the capillary vessels are branches from the spiral modiolar artery They run centrifugally over the spiral lamina (Fig 3) Each RAL supplies a rather small segment When RAL reach the vessel of the tympanic lip or the vessel of the basilar membrane they turn off squarely in T junctions or build up arcades CVL drain the vessel of the basilar membrane the vessel of the tympanic lip and the limbus vessels and run centripetally over the spiral lamina to empty in the spiral modiolar vein

The vessel of the tympanic lip (VSTL) VSTL is a well supplied and well-drained vessel which is found throughout the cochlea and is typically well injected by contrast It forms one or often two spiral marginal vascular borders in the tympanic lip (Fig 4) There is often a system of vascular loops connecting these two inner spiral vessels in addition to the more central loops in the spiral lamina. In this way the vessels appear to form a capillary net in the spiral lamina which end peripherally in one or two vascular margins of VSTL

The limbus vessels (LVS) LVS make up a capillary network peripherally in the spiral limbus The loops are sparse throughout the entire cochlea Different branches from the same radiating arterioles and collecting venules are provided for the VSTL and VSBM and the LVS and there are no direct connections between those spiral vessels and the LVS The blood supply to the spiral limbus appears poor in the rat in comparison with other mammals

The vessel of the basilar membrane (VSBM) VSBM is not very often seen However large uninjected channels resembling the previously termed avascular channels are almost always seen especially in the basal turn under the tunnel of Corti where VSBM usually lies when present (Fig 5) In cases where the vessel *was* present it was a single injected vessel without branches The non-contrast injected channels themselves appear to be long—extending over a whole turn—with very few supplying radiating arterioles or draining collecting venules At the so-called T junction where supplying and draining radiating arterioles and collecting venules join VSBM one can sometimes see an injected VSBM within a perivascular space running spirally in one direction while a channel without contrast continues spirally in the other direction (Fig. 5) Nothing was ever seen at the T junction to inhibit the injection of Berlin Blue

The vestibular and tectorial membranes are

mall fraction of the whole vesicle population Within the basal lamina only isolated or small groups of 2–3 ferritin molecules were scattered along the circumference of the capillaries. These findings are surprisingly similar to those of the present study However despite the similarities in capillary structure and ferritin transport the transendothelial passage of HRP is vastly different between strial and cortical thymic capillaries Raviola and Karnovsky (1972) demonstrated that the amount of HRP transport across cortical thymic capillaries is comparable to that of ferritin That is from 2–20 min post injection only limited vesicular transport of the enzymatic tracer occurred through endothelia, vascular adventitia was free of peroxidase reaction product Apparently then, the transport of both ferritin and HRP is similar for a given capillary bed provided that the micropinocytotic transport system is the sole means of transendothelial passage The similar transport characteristics of HRP and ferritin agree well with the conclusions of Renkin (1964)

Another blood-tissue barrier has been reported which is similar to the thymic one (Gershon & Bursztajn 1978) The myenteric plexus is supplied with continuous-type capillaries Using HRP Gershon & Bursztajn (1978) demonstrated minimal transport of the tracer presumably by the pinocytotic pathway since intercellular cleft transport was impossible due to tight junctional seals Extravascular accumulation of the HRP reaction product in the myenteric plexus was undetectable as Raviola and Karnovsky reported for cortical thymic tissue However in both tissues—thymus and myenteric plexus—it was concluded that some transport has occurred because perivascular phagocytes contained vacuolated tracer substance

In the present study the ferritin results appear to indicate the existence of a blood-strial barrier which is closer in nature to the blood-thymic and blood-myenteric plexus barriers than to the blood-brain barrier

Another phenomenon which might account for the observed minimal transport of ferritin across strial capillaries is the phagocytotic activity of strial endothelial cells Within these cells were observed large vacuoles and multivesicular bodies containing ferritin similar to those reported by Raviola & Karnovsky (1972) Apparently one means of such vacuole formation was by pinocytotic vesicular fusion since with increasing time larger and denser ferritin containing bodies (presumably lysosomes) were present It appeared that once ferritin conglomerates formed the ferritin was immobilized within endothelia until degraded In essence then the phagocytotic activities of strial endothelia in conjunction with minimal pinocytotic transport and endothelial tight junctions contribute to the formation of a blood-strial barrier at least for molecules the size of ferritin

Previous studies have suggested that intermediate cells may serve as macrophages within the stria. Such cells have been found to have open-mouthed pinocytotic vesicles and intracytoplasmic vacuoles labeled with HRP reaction product after a sufficient interval of time following HRP administration (Duvall et al. 1971 Winther 1971) Also at the light microscopic level Nomura (1961) found that intravenously injected trypan blue was phagocytized by certain strial cells scattered about the capillaries Similarly transported tracer substances within cortical thymic and myenteric plexus blood-tissue barrier systems were incorporated within perivascular phagocytic cells (Raviola & Karnovsky 1972 Gershon & Bursztajn 1978) The possibility exists that in this study some ferritin-like particles within the light cells of experimental animals may be exogenous ferritin that is perhaps the cytoplasm of these cells serves as a sink for transported exogenous ferritin However the fact that such particles were located for the most part, free within the light cell cytoplasmic matrix argues against an exogenous source since macromolecules of this size are not able to enter free cytoplasm. Further it should be noted that there were no obvious differences

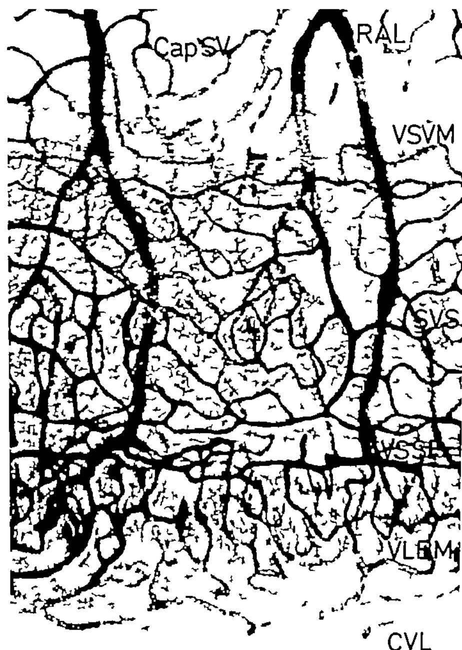


Fig 6 Rat cochlea external wall turn — RAL radiating arterioles supplying SVS and VSSP CapSV capillaries of the scala vestibuli, VSVM vessel at the vestibular membrane SVS stria vascularis VSSP vessel of the spiral prominence VLBM venules at the basilar membrane CVL collecting venules.

avascular which is in agreement with the principal vascular pattern of the other mammals previously examined

External wall

The following vascular structures can be demonstrated in the external wall

Scala vestibuli radiating arterioles RAL capillary net of the scala vestibuli CapSV vessel at the vestibular membrane VSVM

Radiating arterioles (RAL) RAL originate from the spiral modiolar artery and supply all the capillary areas in the external wall (Fig 3) In general RAL are large vessels which run a serpentine centrifugal course before they straighten out and ramify RAL seem to maintain the same calibre from base to apex The most frequently observed pattern of arterial supply of the stria vascularis is for the RAL to run external to the strial bed and then connect directly to the basal marginal stria vessel The basal marginal vessel usually was more prominent than other capillaries in the stria vascularis More RAL branches seem to supply

ZUSAMMENFASSUNG

Enzymatische Tracermethoden für die Erforschung normales und pathologisches Kapillartransports der Stria stellen verschiedene Probleme vor. Die Verwendung opaker Elektronentracer kann viele von diesen Problemen verhüten. Eisendextran (mol. Dm. 20–70 Å) und Ferritin (mol. Dm. 110 Å) wurden intravenös eingespritzt, und die Mäuse wurden nach Zwischenzeiten von ½, 1, 2, 5 und 24 Stunden geopfert. Die Eisendextran-Ergebnisse waren ungewöhnlich, weil der Tracer von ½ bis 5 Stunden nach der Einspritzung in der zytoplasmatischen Matrize der Endothelien zugegen war; aber gegen die 24. Stunde war er ausgeräumt worden. Kein transendothelialer Austausch wurde bemerkt. Die Ferritin-Ergebnisse widersprachen vorhergehenden Ergebnissen, die Meerrettichperoxidase benutzten. Der Transport von Ferritin war ungeachtet der verstrichenen Zeit minimal. Nur ein paar Moleküle wurden um die Basalkapillarlamina bestreut. Die quer durch Kapillaren übergeführten Moleküle wurden anscheinend mittels des makropinozytotischen Systems übergebracht. Die Ergebnisse deuten eine Blut-Stria-Sperre an, die den Blut-Thymus- und Blut-Myenterus-Sperren ähnlich ist. Versuchs- sowie auch Kontrolltiere wiesen Lichtzellen der Stria auf, die Ferritin-ähnliche Partikeln in ihren zytoplasmatischen Matrizen enthielten. Diese Lichtzellen sind wahrscheinlich Zellen des reticuloendothelialen Types. Ferritin könnte nützbar sein, um die auf Gehörpathologien bezogenen Änderungen des Kapillartransports der Stria abzuschätzen.

ACKNOWLEDGEMENTS

The authors wish to thank Prof. J. M. Ventry and Prof. O. R. Anderson, Teachers College, Columbia University for their support of this work. Portions of this work were taken from the dissertation submitted by J. S. S. in partial fulfillment of the requirements of the Ph.D. degree, Columbia University 1978. This work was supported in part by Grant-in-Aid of Research from Sigma XI, The Scientific Research Society of North America and by grant from the Deafness Research Foundation. Thanks to Celeste Conway for help in the preparation of this manuscript.

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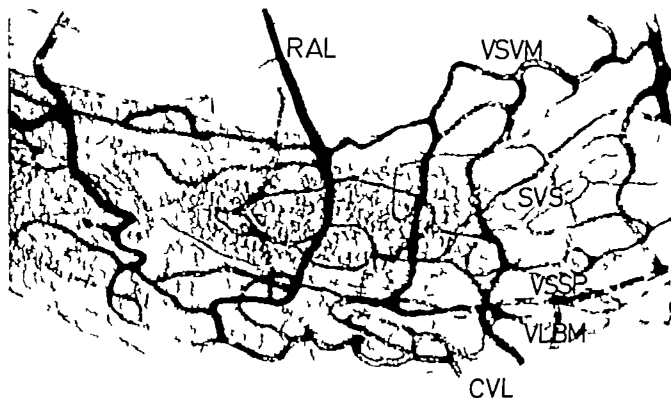


Fig 8 Rat cochlea turn 3 Apical simplification *RAL* radiating arterioles *VSVM* vessel at the vestibular membrane *SVS* stria vascularis *VSSP* vessel of the spiral prominence *VLBM* venules at the basilar membrane *CVL* collecting venules

veloped in terms of number of loops in the middle turns There is a simplification of the SVS in the third turn In the basal turn the width of the stria is decreased and the loops are more tightly packed

The vessel of the spiral prominence (VSSP) Parallel and basal to the stria vascularis runs VSSP (Figs 6 and 7) It is supplied by separate branches of the radiating arterioles of the scala vestibuli VSSP is made up of spirally running segments which are irregular i e long and short segments are interspersed Very often VSSP ramifies into a double vessel VSSP is often drained by a short branch which first travels above the spirally running segment and then loops downward toward the scala tympani into the collecting venule For short distances there can be an absence of the VSSP VSSP is approximately of the same calibre as the basal marginal stria vessel There seems to be no difference with respect to vessel calibre apically and basally

Arterio-venous anastomoses (AVAS) External to the stria vascularis and the vessel of the spiral prominence there are connections between the radiating arterioles in the scala vestibuli and the collecting venules in the scala tympani These vessels consequently offer a by pass possibility where the arterial blood in the scala vestibuli can be shunted over directly to the venous blood in the scala tympani

Scala tympani Venules at the basilar membrane VLBM collecting venules scala tympani CVL ST

Collecting venules–scala tympani (CVL ST) and the venules at the basilar membrane (VLBM) CVL ST in the rat have a different appearance from many other mammals Most of the venules make up a vascular net in the region of the attachment of the basilar membrane Basally this vascular net has well defined spirally running segments of venules (VLBM) (Figs 6 and 7) This spiral VLBM border is drained by comparatively few ra

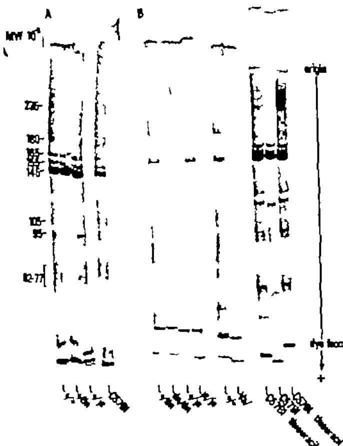


Fig 2 Gels grouped together were subjected to electrophoresis at the same time. Remains of the stacking gels can be seen at the top of the picture, 7% gels stained with Coomassie blue. (A) Normal tectorial membranes from a variety of genetic backgrounds. The molecular weights of the major bands are labelled, and were estimated using the relative mobilities of standard proteins run at the same time as the membrane samples. (B) Tectorial membrane samples from mutants and their normal littermates, and from thiouracil-treated and control C57B1 mice N significant differences in the banding patterns can be detected

method used was based on that described by Saemmli (1970) and Maizel (1971)

A stock solution was added to each sample to bring the final concentration to 2% SDS 0 05 M Tris-HCl (pH 7 0) and 5% -mercaptoethanol and the density was increased by adding about 10% sucrose This was heated to 100°C for two minutes to ensure denaturation of the proteins The numbers of samples examined were as follows C57Bl – 18 C57Bl + thiouracil – 13 je/je – 6 +/je – 7 dn/dn – 6 +/dn – 7 Va/+ – 6 +/+ – 7

Gels were cast in glass tubes 9 cm long, with an internal diameter of 0.27 cm, and were prepared by standard procedures as described by Davis (1964) and Ornstein (1964) Seven per cent acrylamide running gels and 3% stacking gels were used both containing 0 1% SDS and with an acrylamide to bisacrylamide ratio of 30 0.8

The discontinuous buffer system of Laemmli (1970) was used with Tris-HCl in the running gel and Tris-glycine at the electrodes The electrode buffer consisted of 25 mM Tris 192 mM glycine and 0 1% SDS and its pH was 8.5 Electrophoresis was carried out with a constant voltage and an initial current of 1.5 mA per gel and was stopped after about two hours when the tracking dye reached a set point. The gels were then removed from their tubes and fixed and stained with Coomassie blue as described by Fairbanks et al (1971)

Standard proteins of known sub-unit molecular weight were used to calibrate the gels and the molecular weights of the proteins in the membrane samples were obtained by com-

net is formed The stria vascularis is unusually well developed all the way to the extreme basal end in the rat cochlea and better developed than in the other mammals studied so far (Fig 9) The capillary areas at the basal end are drained by collecting venules which empty into the vein of the round window

DISCUSSION

The reason for studying the vascular anatomy of the rat cochlea is the frequent use of this animal in experiments concerning hearing research There has been a lack of systematic investigation of the complete cochlear vasculature in this animal The contrast injection technique using Berlin Blue has proved to be a satisfactory method for studying vascular anatomy It would be misleading however to suggest that the technique proceeds without difficulty 2 5% Berlin Blue in distilled water alone gave a poor and uneven injection A better more even injection was achieved by dissolving the 2 5% Berlin Blue contrast powder in an 8% citric acid solution Insertion of the plastic tube with perfusion and contrast was more easily performed in animals of 250 g than in smaller animals Some vessels stained better than others which suggests that these routes offer less resistance to circulation than the less well injected vessels do

The vascular supply in the mammals thus far studied by us—man rhesus monkey rabbit chinchilla guinea pig and rat—seems to be strikingly similar Each of these animals however has particular anatomical details usually making it fairly easy to differentiate its cochlear vasculature from other mammals In the spiral lamina the vessel of the tympanic lip in most mammals can be described as a single vessel bordering the tympanic lip being supplied and drained in an arcadic fashion In the rat the vessel of the tympanic lip also forms a spiral vascular border in the tympanic lip but a parallel vessel or vascular loop forming a capillary net may also be found running more centrally In this respect the rat vessels are similar to the open and curved vascular arcades found in the rabbit but different from other mammals

The vessel of the basilar membrane was only occasionally injected by contrast Most of the time it appeared as a channel without red blood cells and without injected contrast. So far the vessel of the basilar membrane has only regularly been identified in the guinea pig and in man As in the rat it can be demonstrated by contrast in short sections in the chinchilla but not at all in the rabbit Many authors have discussed the possible importance of the vessel of the basilar membrane for the oxygen supply to the organ of Corti It is well known that this vessel is large during embryonic life much larger than in adult life It appears then that the vessel of the basilar membrane may be important for the development of the organ of Corti but less so for the maintenance of its oxygen supply during adult life since it is often missing The significance of the empty channels cannot be evaluated with the present technique Probably it is a remnant of a blood vessel It might be argued that in case of a particular need for blood supply to the organ of Corti these channels could regain their red blood cell circulation and function as blood vessels

The external wall is the region which appears to be most similar in different mammals with easily identified supplying and draining vessels to well-defined capillary areas The most obvious difference between the rat and other mammals is found in the collecting venules in the scala tympani In the rat external wall there is a rich net of collecting venules in the region of the attachment of the basilar membrane but as the venules continue basally they often form a sparse radially running network which is drained by spiral segments of larger collecting venules

In one animal a "suspension vein was found running from the modiolus through the perilymph to the collecting venules of the scala tympani in the external wall The suspension vein appeared to be of larger calibre

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THE ELASTIC PROPERTIES OF THE TYMPANIC MEMBRANE SYSTEM IN DIVERS AND NON DIVERS

A. Ivarsson Ö Tjernström and R. Uddman

From the Department of Otolaryngology University of Lund Malmö General Hospital Malmö Sweden

(Received April 19 1979)

Abstract The volume/pressure relationship of the tympanic membrane system was studied in 24 scuba divers The results were compared with the results obtained from 96 non-diving subjects Among persons with a good function of the Eustachian tube divers had an increased mobility of the tympanic membrane system as compared to non diving subjects Divers with reduced function of the Eustachian tube did not differ from the non-diving subjects with reduced tubal function. Factors that might influence the volume/pressure relationship of the tympanic membrane system are discussed.

The past two decades have seen a great increase in the number of people engaged in commercial as well as recreational diving In 1965 it was estimated that five million people in the United States were utilizing scuba diving equipment. This growing interest in scuba diving has increased the number of preferably young people diving with limited training and often too little knowledge of the risks involved Thus, it seems to be of great importance to expand our knowledge of the physiology as well as pathophysiology of the ear in diving.

Otologic problems related to exposure to compressed gas environments are most often an effect of inadequate equalization between the middle ear and the ambient pressure Diving implies rapid large and frequent changes in ambient pressure and it can be assumed that inability to equilibrate middle ear pressure with ambient pressure might affect the elastic elements of the tympanic membrane system i.e the tympanic membrane, ossicles ligaments and middle ear muscles The elastic properties of the tympanic membrane system can be determined by its volume/pressure relationship

Studies on normal subjects without diving experience have revealed a relation between the elastic properties of the tympanic membrane system and the function of the Eustachian tube Subjects with a reduced tubal function had a more mobile tympanic membrane (Elner et al. 1971*c*) This was considered to be caused by an inability to keep the middle ear pressure equal to ambient pressure With these results in mind we found it of interest to study to what extent repeated barotrauma might cause changes in the elastic properties of the tympanic membrane system in a group of experienced scuba divers

The aim of the present study was (1) to compare the elastic properties of the tympanic membrane system in divers and non-divers and (2) to find out if there is a relation between the elastic properties of the tympanic membrane system and the Eustachian tubal function in divers also

METHOD

Quantitative studies of the pressure regulating capacity of the Eustachian tube were made

This study was supported by the Swedish National Defense Research Institute, project No. H352.

Acta Otolaryngol 89 12–26 1980

AN EVALUATION OF NORMAL STRIAL CAPILLARY TRANSPORT USING THE ELECTRON-OPAQUE TRACERS FERRITIN AND IRON DEXTRAN

J Santos Sacchi[1] and William F Marovitz[2]

From the [1]Center for Biomedical Education a d [2]Dept of Otolary gology Mou t Sinai School of Medicine City University of Ne York New York

(Received March 14 1979)

Abstract Enzymatic tracer techniques to study normal and pathologic strial capillary transport pose various problems The use of electron opaque tracers can circumvent many of these problems Iron dextran (mol diam 70–70 Å) and ferritin (mol diam 110 Å) were injected intravenously and the mice sacrificed at intervals of ½ 1 2 5 and 24 h The iron dextran results were unusual in that from ½ to 5 h after administration the tracer was present within the cytoplasmic matrix of endothelia, but by 4 h it had been cleared out. No transendothelial exchange was noted The ferritin results were in conflict with previous results using horse-radish peroxidase Transport of ferritin was minimal regardless of time sacrificed No more than a few molecules were scattered about the capillary basal lamina. Those molecules transported across capillaries were apparently delivered by means of the micropinocytotic system The results suggest a blood-strial barrier similar to the blood-thymic and blood-myenteric barriers Experimental as well as control animals exhibited strial light cells which contained ferritin-like particles within their cytoplasmic matrices These light cells are probably reticulo-endothelial type cells Ferritin may be useful to gauge strial capillary transport alterations associated with auditory pathologies.

The functional role of the stria vascularis which includes the production of the endocochlear DC potential and ionic composition of the endolymph is important for normal inner ear function (Honrubia & Ward 1969 Konishi et al 1966) The stria vascularis lines the lateral wall of the cochlear duct and is composed of three cell layers termed marginal intermediate and basal (Smith 1957) The tissue is effectively a separate compartment sealed off from the endolymphatic space and spiral ligament by zonulae occludens which occur between the cell membranes of abutting marginal cells and abutting basal cells (Jahnke 1976) This tight junctional seal around the stria limits the nutrient inflow from neighboring sources therefore the high strial metabolic requirements (Chou & Rodgers 1962) must be met essentially by intrinsic capillary networks (Reale et al 1975) Thus the permeability functions of the strial capillaries are paramount when considering the survival and function of the strial cells accordingly strial capillary permeability is important for normal cochlear function

Tracer molecules have been used to map the transport of materials through strial capillaries (Yamamoto & Nakai 1964 Duvall et al 1971 Winther 1971 Gorgas & Jahnke 1974) Tight junctions have been found to exist between strial endothelial cells as have been found to exist between capillary endothelia of most CNS tissue (Duvall et al 1971 Gorgas & Jahnke 1974 Reese & Karnovsky 1967) Tight junctional seals are known to prevent the passage of lipid insoluble materials through intercellular clefts Therefore passage of lipid insoluble materials through the capillaries of the stria vascularis appears to be limited to the micropinocytotic vesicular system (Duvall et al 1971 Gorgas & Jahnke 1974) By means of the vesicular system transport of the tracer molecule horseradish peroxidase (HRP mol wt 43 000 mol diam 55 Å) is very rapid As early as 1–2 min following HRP injection Duvall et al (1971) observed the HRP reaction product distributed through strial capillary basal lamina In muscle however more than

Acta Otolaryngol 89 37–42, 1980

VIBRATION MEASUREMENT OF THE HUMAN TYMPANIC MEMBRANE—IN VIVO

O J Lokberg K Hogmoen and T Gundersen[1]

From the University of Trondheim Norwegian Institute of Technology Physics Department and [1]Department of Ear Nose Throat Regional Hospital, Trondheim Norway

(Received March 12, 1979)

Abstract The vibration patterns of the human eardrum in vivo have been recorded by time average electronic speckle pattern interferometry—ESPI. The necessary stability was achieved by shortening the exposure time of each TV frame. The amplitude- and phasedistribution was mapped across the drum by phase-modulation techniques which also could show the vibrations in slow motion. By photoelectric detection of the TV image intensity amplitudes down to 2 nm could be measured. Preliminary results are presented.

Many investigations of the mechanical component of sound transmission of the middle ear have been carried out on human temporal bone preparations Conclusions drawn from such experiments are uncertain as it is not known to what degree the behaviour of the mechanical system in living man corresponds to observations made from temporal bone preparations

Experiments in vitro with human temporal bone provide no information about the effect of the muscles of the middle ear on the mechanics of movement

The sizes of the amplitudes produced by the tympanic membrane and the ossicular chain are of importance in understanding the role this part of the mechanical system plays in the sensibility of the ear

Wilska (1935) measured the movement on the tympanic membrane at the threshold of hearing in one patient. This measurement was obtained by connecting the tympanic membrane to a thin rod leading from the movable coil in a dynamic loudspeaker In this way he was able to cause the tympanic membrane to record the rod's vibrations The apparatus was calibrated by means of a microscope but the calibration was possible only for frequencies up to 270 Hz. His results for frequencies above 270 Hz were obtained by extrapolation and are therefore in our opinion unreliable

Today we have in holography a method which may be used for measurements of the drum and part of the malleus in living man

HOLOGRAPHY

Holography is in principle a means of using laser light to create a unique photographic recording of the light reflected from an object without the use of a lens This photographic recording is called a hologram The hologram bears no resemblance to the image of the object but appears to be an unrecognizable pattern of stripes and whorls when examined under great magnification. However when the hologram is illuminated by light from a laser the light is organized as by magic into a three dimensional representation of the original object The optical illusion is so realistic that it is impossible to decide whether the object is real or not without grasping for it physically

An ordinary photographic image records the variations in intensity of light reflected from the object producing dark areas where less light is reflected and bright areas where more light is reflected Holography however records not only the intensity of the object light but also its phase In this connection the phase

Table I *Number of mice per experimental condition*

	Post injection sacrifice time (h)				
Tracer	½	1	2	5	24
Ferritin	6	4	3	4	7
Iron dextran	2			2	2

Many non-enzymatic electron-opaque tracers have been used to study capillary transport at the ultrastructural level In particular ferritin has been successfully used to delineate certain aspects of transendothelial exchange in a variety of tissues (Farquhar et al 1961 Bruns & Palade 1968 Clementi & Palade 1969) There are many reasons why ferritin may be considered an ideal tracer (Bruns & Palade 1968) especially for strial capillaries They include (1) ferritin (mol wt 460 000 mol diam 110 Å) is the size required for a probe molecule of the large pore transport system which is reportedly the only system in strial capillaries (2) it is a biological substance similar in nature and in size to most plasma proteins (3) high blood concentrations are well tolerated by experimental animals (4) individual molecules can be identified within ultrathin sections and therefore a direct measure of transport is possible (5) ferritin is resistant to lysosomal hydrolases (Drysdale & Munro 1966 Coffey & DeDuve 1968) and therefore may be useful in pathologic conditions

The advantage which the electron opaque tracer molecule ferritin may provide over enzymatic tracers warranted its evaluation In addition because the iron dextran results reported by Yamamoto & Nakai (1964) are in conflict with other strial capillary permeability studies a reevaluation was judged necessary Therefore it was the purpose of this study to evaluate strial capillary permeability with the electron-opaque tracer molecules ferritin and iron dextran

MATERIALS AND METHODS

Tracer substances

Iron dextran (Imferon Lakeside Labs Milwaukee Wis) was dispersed in physiological saline with 0 5% phenol as a preservative and contained the equivalent of 50 mg elemental iron per ml

A 10 ml stock solution of 100 mg of cadmium crystallized ferritin per ml of physiological saline was obtained (Nutritional Biochemicals Cleveland Ohio) To remove the cadmium the solution was dialysed against 3 7% ethylenediamine tetraacetic acid (EDTA) in 0 2 M phosphate buffer pH 7 0 at 0–4°C for two changes (48 h each) Following two additional changes in buffer alone (24 h each) the ferritin solution was dialysed against physiological saline (24 h) and then passed through a 0 45 micrometer millipore filter into a sterile 10 ml injection vial

Animals

Young adult male mice BALB c/J (Jackson Labs Bar Harbor Maine) ranging in weight from 15 to 20 g and showing positive Preyer reflexes to finger snaps were used The animals were screened for middle ear infection by direct examination during temporal bone removal

Methods

All animals received subcutaneous injections of sodium pentobarbital 2 mg/20 g body weight prior to injection of tracer Anesthesia was reached within 15 min and lasted for 2–3 h for those animals allowed to recover Under anesthesia either the jugular vein or the inferior vena cava was exposed and was injected with 1 ml per 100 g body weight of ferritin or iron dextran solution The injection was delivered through a 30 gauge needle over a period of 1–2 min Animals were sacrificed by decapitation 1/2 1 2 5 or 24 h after injection Twelve control animals received anesthetic injection only and were sacrificed immediately after induction The number of

Acta Otolaryngol 89: 37–4? 1980

VIBRATION MEASUREMENT OF THE HUMAN TYMPANIC MEMBRANE—IN VIVO

O. J. Løkberg[1], K. Høgmoen and T. Gundersen[2]

From the University of Trondheim, Norwegian Institute of Technology, Physics Department and [2]Department of Ear Nose Throat, Regional Hospital, Trondheim, Norway

(Received March 1., 1979)

Abstract. The vibration patterns of the human eardrum in vivo have been recorded by time average electronic speckle pattern interferometry—ESPI. The necessary stability was achieved by shortening the exposure time of each TV frame. The amplitude- and phasedistribution was mapped across the drum by phase-modulation technique which also could show the vibrations in slow motion. By photoelectric detection of the TV image intensity amplitudes down to 2 μm could be measured. Preliminary results are presented.

Many investigations of the mechanical component of sound transmission of the middle ear have been carried out on human temporal bone preparations. Conclusions drawn from such experiments are uncertain, as it is not known to what degree the behaviour of the mechanical system in living man corresponds to observations made from temporal bone preparations.

Experiments in vitro with human temporal bone provide no information about the effect of the muscles of the middle ear on the mechanics of movement.

The sizes of the amplitudes produced by the tympanic membrane and the ossicular chain are of importance in understanding the role this part of the mechanical system plays in the sensibility of the ear.

Wilska (1935) measured the movement on the tympanic membrane at the threshold of hearing in one patient. This measurement was obtained by connecting the tympanic membrane to a thin rod leading from the movable coil in a dynamic loudspeaker. In this way he was able to cause the tympanic membrane to record the rod's vibrations. The apparatus was calibrated by means of a microscope but the calibration was possible only for frequencies up to 270 Hz. His results for frequencies above 270 Hz were obtained by extrapolation and are therefore in our opinion unreliable.

Today we have in holography a method which may be used for measurements of the drum and part of the malleus in living man.

HOLOGRAPHY

Holography is in principle a means of using laser light to create a unique photographic recording of the light reflected from an object without the use of a lens. This photographic recording is called a hologram. The hologram bears no resemblance to the image of the object, but appears to be an unrecognizable pattern of stripes and whorls when examined under great magnification. However when the hologram is illuminated by light from a laser the light is organized as by magic into a three dimensional representation of the original object. The optical illusion is so realistic that it is impossible to decide whether the object is real or not without grasping for it physically.

An ordinary photographic image records the variations in intensity of light reflected from the object producing dark areas where less light is reflected and bright areas where more light is reflected. Holography however records not only the intensity of the object light but also its phase. In this connection the phase

animals per experimental condition is noted in Table 1

Cochleae were fixed either in 1% osmium tetroxide or 2% glutaraldehyde in 0 2 M Cacodylate buffer pH 7 4 Within 1 min after decapitation the right temporal bone of each animal was excised fractured open and immersed in about 5 ml of primary fixative After 2 h of primary fixation at 0–4°C all cochleae were briefly washed in buffer Those ears fixed in glutaraldehyde were then osmicated for an additional 2 h However from each cochlea of those animals injected with iron dextran pieces of stria were removed while in buffer and were not osmicated At the completion of osmication the specimens were again briefly washed in buffer In ferritin injected animals specimens were selected from basal mid and apical portions of the cochlear duct After the striae were microdissected free from the cochleae in 70% acetone dehydration was completed in graded acetone and the tissue embedded in Epon at 60°C for 24 h

Ultra thin sections were cut on glass and diamond knives using either the Sorvall MT 1 ultramicrotome or an LKB Ultratome Section interference colors ranged around silver Sections were picked up on copper grids and most were examined unstained so that electron dense tracer particles could be unequivocally identified Some sections were stained with lead citrate (Reynolds 1963) Contrast was significantly enhanced by the use of projector field diaphragms with the Zeiss 9S-2 EM

RESULTS

Those animals allowed to recover from anaesthesia had positive Preyer reflexes None of the experimental ears showed evidence of middle ear infection upon direct examination using a dissecting microscope

The ultrastructural appearance of the stria vascularis was typical of the fixation procedure employed (Merck et al 1974 Santos Sacchi 1978*a* and *b*) One noteworthy difference between the two fixation procedures was the decreased concentration of tracer particles within capillary lumina in primary osmium fixed specimens as compared to primary glutaraldehyde fixed tissues

Iron dextran

The plasma concentration of iron dextran decreased over time In the 1/2 h specimens capillaries were observed which contained iron dextran only within endothelial pinocytotic vesicles (Fig 1) However more frequently and in all animals beyond 1/2 h up to 5 h the iron dextran appeared free as well as in vesicles within the cytoplasm of endothelia (Figs 2 and 3) In fact the tracer was occasionally observed within the nucleoplasm of some endothelia By 24 h after injection however the iron dextran was absent from endothelia (Fig 4) The passage of the tracer to the basal lamina was found to be almost nil since rarely were any electron dense particles found in the basal lamina at any time point after injection No tracer was observed within strial cells

Ferritin

The appearance of ferritin within various spaces of the tissues studied was indicative of its passage from the circulatory system into the stria vascularis The results are reported in terms of compartments within which ferritin was observed

Capillary lumen

From 1/2 to 24 h following injection ferritin was present within the capillary lumina of the stria in all areas studied However the concentration of ferritin within the blood plasma varied with time By 24 h after ferritin administration the plasma concentration was considerably lower than its initial level Ferritin was never present within capillary lumina of control animals

Endothelial tunic

From the earliest to the latest time points after administration ferritin labeled pinocytotic

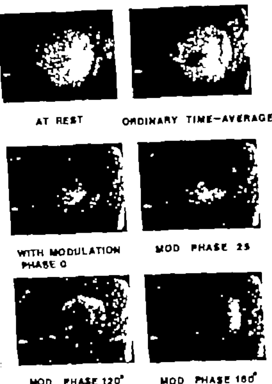


Fig. 4. ESPI recordings of an ear-drum. Upper row: at rest and vibrating at 2400 Hz, 90 dB as recorded by ordinary time-average. The same sound pressure is used in the remaining pictures, but with the modulating mirror vibrating at 0.1 µm and phase settings as indicated.

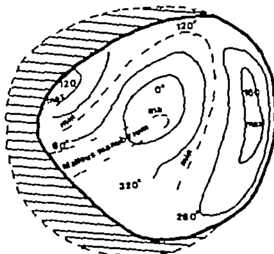


Fig. 5. Sketch indicating the behaviour of the eardrum in Fig. 4 (2400 Hz). The lines are isoamplitude lines with max. and min. amplitudes as given. Phase values are given with numbers (hatched area: not observed part of the drum).

Between 3 and 4.5 kHz there is another peak and the explanation for this may be the increasing of the sound energy in the inner part of the extended meatus as shown by Wiener & Ross (1946). The measurement indicates that the size of the amplitudes of the malleus in living man is considerably greater (ten times ?) than those found in temporal bone preparations. However, we should bear in mind the difference in the SPL measurement procedures in the two cases.

Fig. 4 shows ESPI interferograms photographed off the TV-monitor. The first is recorded with the membrane at rest, the others are recorded at 2400 Hz—90 dB SPL. No. 2 is recorded without reference wave modulation. The manubrium can be observed where the reflectivity and the fringe contrast are better. In the other areas of the membrane, the poor fringe contrast makes it difficult to observe any fringes.

The remaining four pictures are recorded with phase modulation and with different phase settings (0°, 25°, 120°, 160°) which make the zero order fringe move about on the membrane. This is much better observed *live* on the TV monitor, using a continuous phase shift, than in the still photographs.

In Fig. 5, what is believed to be the vibratory behaviour of the tympanic membrane at 2400 Hz was sketched as an example. At this frequency the motion is complex with gross variations in vibration phase. In the other drum of Fig. 3 the movement was much more uniform with small phase variations. The amplitude of the manubrium tip was, however, quite equal.

ZUSAMMENFASSUNG

Die Schwingungen des lebenden Menschentrommelfells sind registriert worden, mittels Durchschnittszeit elektronischer Speckle-Interferometrie – ESPI. Die notwendige Stabilität wurde bei Verkürzung der Expose-

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Acta Otolaryngol 89: 43–52 1980

THE ROLE OF MATRIX VESICLES IN THE PATHOGENESIS OF TYMPANOSCLEROSIS

W Mann U N Riede I Jonas and Chl Beck

From Universitäts-HNO-Klinik, Freiburg i. B BRD

(Received January 31 1979)

Abstract In histological and electron microscopical study the authors investigated the pathogenesis of tympanosclerosis Emphasis was placed on the role of extracellular lysosomes (matrix lysosomes) The function of such matrix lysosomes is connected with the two-stage degradation of the connective tissue. This system gets out of control in tympanosclerosis. Inflammatory and immunological processes are suggested to be superimposed on this lysosomal action

Even today the pathogenesis of tympanosclerosis is unclear When reviewing the literature most of the authors assume that some sort of chronic otitis media and a particular predisposition of the patient are a prerequisite (for refs. see Beck & Ebert 1964 Chang, 1969 Sorensen & True 1971) The clinical picture is well known and there is no need for further description.

Describing morphological changes during otitis media, alterations of the epithelium and of the submucosal layers must be regarded separately During tympanosclerosis the superficial epithelium disappears and the submucosal connective tissue is profoundly modified At the ultrastructural level the prominent changes are as follows. marked infiltration with lipid enclaves remarkable changes of the collagenous fibres in structure and number and the presence of cellular debris and of calcium phosphate deposits (Chang, 1969) Preliminary studies have revealed that the cellular debris partially consists of matrix vesicles the lysosomal nature of which could be demonstrated in several connective tissue diseases (Riede & Staubesand 1977)

Considering the fact that lysosomes of the connective tissue cells contain hyaluronidases β-glucuronidases β-N-acetylglucosaminidases, proteases and cathepsins for proteoglycan degradation (Sledge 1968 Dingle 1969 Parakkal 1977) we were interested to study the formal pathogenesis of tympanosclerosis from this point of view

MATERIAL AND METHOD

Histological preparation

Tympanosclerotic plaques of the oval niche from four different patients (2 ♂ and 2 ♀ aged 30–40 years) and tympanic mucosa of 2 patients with chronic purulent otitis media were fixed in 10% buffered formalin at room temperature for 24 h embedded in paraffin wax and sectioned at a thickness of 5 μm

Histochemical preparation and demonstration of oxytalan fibres

The histological slides were stained as follows (Romeis 1968 Fullmer & Lillie 1958 Rannie 1963) Monopersulphate aldehyde fuchsin aldehyde fuchsin periodic acid-Schiff sequence haematoxylin-eosin Taenzer Unna's orcein and Azan's stain

For the monopersulphate aldehyde fuchsin method the tissues were pre-oxidized in a 10% aqueous solution of potassium monopersulphate ('Karoat Degussa, Hanau) The oxidizing properties of potassium monopersulphate are comparable to those of peracetic acid but

often empty on occasion ferritin could be visualized within the basal lamina either just in front of the opening or not far away (Fig 9) Nevertheless there were direct observations of ferritin within vesicles opened upon the luminal and tissue fronts (Figs 7 8 and 9) as well as in those within the endothelial cytoplasm Labeled vesicles within the endothelial cytoplasm were encountered more frequently It cannot be ruled out that ferritin within open mouthed vesicles diffused out during fixation

Another occasional phenomenon was the appearance of tubular invaginations from luminal and tissue surface membranes of endothelial cells (Fig 5) These structures were only encountered in thick portions of endothelia. Ferritin was found within some luminal invaginations but direct connections from luminal to tissue fronts were never observed

There appeared to be a relation between the time after injection and the accumulation of ferritin within vacuoles of endothelia At the earlier time points vacuoles containing ferritin in fairly large amounts were occasionally observed (Fig 8) Rarely large vesicles with high concentrations of ferritin were seen open to the plasma front as if in the process of loading (Fig. 5) With increasing time larger and denser cytoplasmic vacuoles were noted within which individual ferritin granules could be observed (Fig 11) Pinocytotic vesicles labeled with ferritin were seen fused with large ferritin containing vacuoles (Fig 8) or with each other (Fig 5) Multi vesicular bodies were also seen that is single membrane vacuoles within which were found labeled as well as unlabeled pinocytotic like vesicles (Fig 10)

Ferritin was neither seen in pinocytotic or other structures within endothelia of control animals nor was it observed free in the endothelial cytoplasm of experimental or control animals

Basal lamina and intercellular spaces

The basal lamina was not heavily infiltrated with ferritin molecules at any time point after injection In the earlier specimens observed molecules were either absent or present at extremely low concentrations singly scattered about the perimeter of the capillary (Figs. 5 and 8) No accumulation of ferritin within the basal lamina at particular points around the capillary was noted save one instance This occurred in a capillary of an animal sacrificed 1/2 h after injection (Fig 12) An apparent leak within a discrete portion of a single capillary allowed a relatively large quantity of ferritin to escape into the basal lamina This was the only occurrence of this kind in all specimens studied and adjacent strial capillaries within the same section did not exhibit this profuse transendothelial passage of ferritin This phenomenon was clearly not typical of normal strial capillary function

In the 5 and 24 h specimens accumulation of ferritin appeared slightly greater around some capillaries than in earlier specimens However this was not the rule since other capillaries at the same time periods showed little or no ferritin present within the basal lamina. The overall picture revealed that ferritin labeling of the basal lamina was variable at all time points studied and that labeling even at its greatest was scant

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Fig 7 Large arrow indicates numerous labeled pinocytotic vesicles within endothelium Smaller arrow indicates open-mouthed vesicle upon luminal front which appears to have ferritin molecules within neck portion of vesicle 1 h post injection. Osmium fixed Stained with lead citrate Mid portion of cochlear duct ×97 325

Fig 8 Note numerous open-mouthed pinocytotic vesicles upon tissue front Medium arrow indicates a ferritin labeled one A ferritin molecule can be seen in the basal lamina (small arrow) Within the endothelial cell a pinocytotic vesicle can be seen fused with a large vacuole containing numerous ferritin molecules (large arrow) 5 h post injection Glutaraldehyde and osmium fixed. Unstained Basal portion of cochlear duct ×88 635

Fig 9 Large arrow indicates open-mouthed pinocytotic vesicle containing two ferritin molecules on luminal front A ferritin molecule can be seen at the opening of pinocytotic vesicle at the tissue front (medium arrow) Small arrows indicate ferritin molecules within basal lamina h after injection Osmium fixed Unstained Mid portion of cochlear duct ×97 470



Fig. 3b. Osmophilic degeneration of the ground substance (↑) in between oxytalan fibrils (ox) (×14 400). *Inset:* Magnification, ×23 600.

peared isolated or clustered calcified or uncalcified extracellular or intracellular (Fig. 5*a*, *b*).

Cells. The dominant cell type belonged to the cell group of fibroblasts. Most of them were irregular-shaped and some were degenerating. Characteristics were: The increased osmophilic cytoplasm, the accumulation of cytoplasmatic vesicles in the peripheral cytoplasm and the increased number of cytoplasmatic processes. No giant cells (=macrophages) were observed.

Similar findings were seen to a minor degree in patients with chronic purulent otitis media, but never in healthy individuals.

DISCUSSION

The prominent feature of the tympanosclerosis is a disturbance of the extracellular space in the submucosa. This means an alteration of the three main components. Elastin, collagen and proteoglycans. As there is an interaction between the composition of the extracellular space and the cellular components the pathogenesis of tympanosclerosis may be due either to a disturbance in the intercellular space or to a disturbance at the cellular level.

Formal pathogenesis

With regard to the formal pathogenesis, the most marked alteration is linked to the fibrous

Ferritin molecules within the intercellular spaces of the stria vascularis were not observed and presumably the size of the molecule limited its penetration into such spaces Ferritin was not seen within the basal lamina of control animals

Strial cells

Ferritin was not seen within the marginal cells However large accumulations of electron dense particles closely resembling ferritin in size and shape were often found in specific cell processes of light cells (Fig 6) In fact the particles within these cells and the injected ferritin molecules appeared indistinguishable The particles were found in high concentration free in the cytoplasm often accompanied by lysosomes containing the same particles This phenomenon was found throughout all time periods studied and *in control animals as well* Further description and analysis of these cells will be the subject of another report (Santos Sacchi and Marovitz in preparation) No pinocytotic uptake of injected ferritin by these light cells was seen although light cells occasionally possessed open-mouthed vesicles on the basal lamina. Also no spectacular accumulation of ferritin within vacuoles was ever observed in these cells There were no apparent differences in cytoplasmic ferritin-like concentration between experimental and control animals

DISCUSSION

Iron dextran

The iron dextran results were perplexing Iron dextran was present within strial endothelial cytoplasm from 1/2 to at least 5 h but was apparently absent by 24 h This did not seem to be a fixation artifact It was concluded that the tracer initially entered the endothelia by means of pinocytotic uptake since in some 1/2 h specimens and in the endothelia of a control animal (killed immediately after injection) iron dextran was present within vesicles and absent within the cytoplasmic matrix The occurrence of iron dextran within the cytoplasmic matrix of endothelia is difficult to explain since it is generally acknowledged that macromolecules have no route by which to enter the membrane protected cytoplasm Although molecules the size of iron dextran are unable to pass through intact membranes similar observations of free intracytoplasmic iron dextran following administration have been reported Richter (1959) after injecting iron dextran intraperitoneally studied the fate of that substance within liver and spleen After 1 h he found accumulations of iron dextran within macrophages and sinusoidal endothelia of both organs There were non membrane bound aggregates of iron dextran within the cytoplasmic matrices as well as numerous free particles This is similar to the results of the present study However in Richter's study by the fouth hour following injection aggregates were enclosed by limiting membranes Further iron dextran was discernible within the cells up to 6 days after administration In this regard Richter s results differ from the present study because iron dextran was absent from 24 h However it should be recalled that in the only previous study of iron dextran permeability in strial capillaries (Yamamoto & Nakai 1964) the molecules remained within endothelia reportedly within vacuoles for 5 h but by 24 h and beyond iron dextran was absent from endothelia. It seems that strial endothelia have an ability to remove or possibly metabolize iron dextran so that by 24 h very little or none remains observable Yamamoto and Nakai reported no transendothelial passage of iron dextran into the stria. Although this appears to be the case for the present study as well the possibility exists that passage of some degraded form of the original molecule occurred which could not be visualized electronmicroscopically Richter s experiment suggests that mouse cells may be able to metabolize the partially depolymerized dextran sufficiently to abolish the colloidal stability of the iron dextran preparation

ment and to an alteration of the proteoglyans concerned with fibrillogenesis (Tyberg 974)

2. There is calcification of the connective issue Type I matrix vesicles contain alkaline hosphatase which cleaves inorganic pyrohosphate inhibitors of calcification as uncouplers in the cell membrane and contain ATPases for the active transport of calcium nto Type II matrix vesicles (Anderson 1976) First crystallization of calcium apatite takes place in these matrix vesicles Type II (Höhing et al. 1976)

Considering the pathogenesis of tympanosclerosis from the clinical point of view the disease may be affected positively by antinecrotic agents with lysosomal stabilizing effects such as cortisone phenylbutazone antihistamines (Weissmann 1969) and Flavichromin (Schole et al 1978 Jonas & Riede 1979) However this statement has no clinical relevance as long as there is no possibility of detecting the initial phase of tympanosclerosis. This is the aim of further experimental and clinical investigations

ZUSAMMENFASSUNG

In einer histochemischen und ultrastrukturellen Arbeit werden die pathologischen Bindegewebsveränderungen bei der Tympanosklerose analysiert Die Autoren entwickeln ein Konzept zur formalen Pathogenese dieser Erkrankung. Eine wichtige Rolle wird den extrazellulär liegenden Lysosomen (= Matrixvesikel) zugesprochen, die am „2 Schritt-Abbau" des Bindegewebes entscheidend mitbeteiligt sind Für die Tympanosklerose postulieren die Autoren eine Dysfunktion dieses Systems, verbunden mit einer verstärkt lysosomalen Aktivität Dieser autophagolytischen Reaktion scheinen sich entzündliche und immunologische Prozesse aufzulagern

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Of course diffuse iron in ionic form or bound in low concentrations to carrier substances cannot be seen (Muir & Goldberg 1961)

Clearly then the use of iron dextran as an electron opaque tracer molecule for strial capillary transport is obviated by the possibly unique treatment it receives by strial endothelia which apparently results in the intra cytoplasmic sequestration and eventual removal of iron dextran

Ferritin

Although ferritin transport across strial capillaries had not been evaluated prior to the present study HRP transport had been examined The transport of the enzyme through strial vessels is very rapid Within a few min after HRP injection the basal lamina and intercellular spaces are densely stained with reaction product (Duvall et al 1971 Winther 1971) These results are more similar to those obtained with muscle capillaries than to those obtained with CNS capillaries except that intercellular cleft transport of HRP is reportedly absent in strial capillaries However the results of the present study which suggest minimal capillary transport indicate that the permeability function of the strial capillaries is more similar to that of the CNS vessels Thus even by 24 h post injection transport of ferritin across endothelia is scanty as determined by basal lamina labeling Although transport of lipid insoluble substances certainly increases as molecular weight decreases the evidence evaluated by Renkin (1964) suggests that transport rates of molecules in excess of about 60 000 Daltons remains constant If the micropinocytotic transport system is the sole means of transendothelial passage of lipid insoluble material within the stria, then the passage of HRP (whose molecular weight is close to Renkin s cut off size) should not be drastically different from that of ferritin This may especially be true if as some researchers believe (Balint & Nagy 1971) HRP binds to B-globulin and IgG within plasma thereby increasing the particle size and bringing it closer to the size of ferritin Thus by size comparison it is difficult to explain the similarity of HRP results and the dissimilarity of ferritin results between strial and muscle capillaries Certainly the very small number of labeled strial capillary pinocytotic vesicles could account for the smaller amount of ferritin transport in strial as compared to muscle capillaries Moreover the dissimilarity of transport suggests that some other factor may play an important role in the rapid transport of HRP across strial endothelia It is here that the established ototoxicity of HRP may come to bear (Ross et al 1977) Perhaps the transport of HRP is rapid in strial capillaries because the HRP *itself* alters strial capillary permeability Such a hypothesis would explain the similarity of HRP results in muscle and strial capillaries and would not conflict with the ferritin results

Research on the permeability of continuous type capillaries in other tissues also leads to the conclusion that the strial HRP results are unusual i e in the light of this study s findings with ferritin Raviola & Karnovsky (1972) evaluated thymic capillary transport using a variety of enzymatic tracers including HRP They also used the tracer molecule ferritin so that it is possible to compare the permeabilities of both HRP and ferritin in the same capillary system One must note however that the thymus contains both continuous and fenestrated capillaries For the present purpose only the continuous capillaries permeability functions are of significance since these capillaries are structurally comparable to strial capillaries Interestingly these continuous capillaries located within the cortical areas of the thymus present a blood-thymic barrier As in the stria and CNS circumferential tight junctions halt extravascular leakage through endothelial intercellular clefts At all time periods from 1–24 h after ferritin injection minimal amounts of the electron opaque tracer had traversed the thymic capillary endothelia. Labeling of pinocytotic vesicles was variable but represented a very

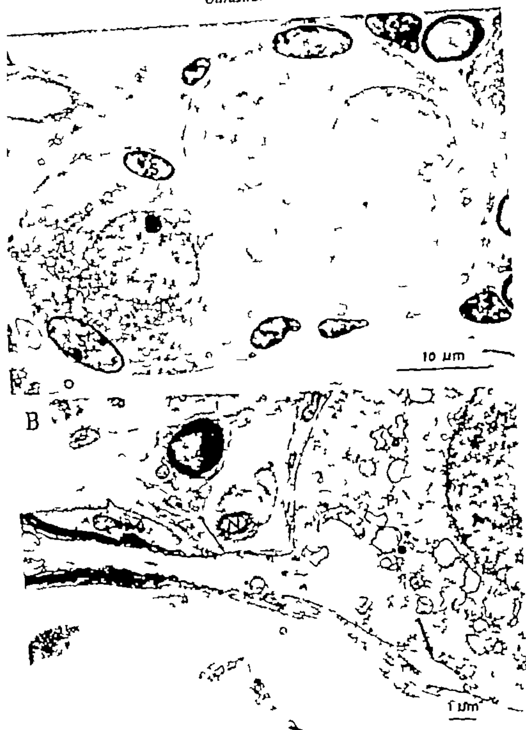


Fig. 2 (A) An electron micrograph of the typical large unmyelinated neurons. The lower perikaryon is surrounded by two satellite cells (*S*) and shows a long axonal process. (B) A dendritic process of the large unmyelinated neurons showing myelination a short distance from the perikaryon (*P*). Note the lack of Schwann cell covering at the node of Ranvier (small arrow) and nearby nerve fiber (*N*). The light elongated structure on the lower right corner (large arrow) is either another process of the satellite cell or an evagination of the perikaryon.

between control and experimental animals in regard to concentration of intracytoplasmic ferritin-like particles Nevertheless within the cytoplasm of liver cells there is de novo apoferritin production within a few h following administration subsequently iron sequestration by apoferritin occurs producing ferritin (Drysdale & Munro 1966) In the present study since the injected tracer contains iron the de novo production of endogenous ferritin by experimental strial light cells might occur if indeed they phagocytized transported exogenous ferritin The problem is made more difficult because the horse spleen ferritin tracer is very resistant to lysosomal breakdown (Coffey & DeDuve 1968) Thus although ferritin may accumulate within lysosomes elemental iron may not be able to enter the cytoplasm because of the hydrolytic resistance of the protein On the other hand there is some evidence that the iron may enter and leave the apoferritin shell without protein disintegration (Mazur & Carleton 1963) In this regard it is noteworthy that in other permeability studies using ferritin as a tracer (Bruns & Palade 1968 Raviola & Karnovsky 1972) after 24 h perivascular macrophages did exhibit intracytoplasmic ferritin granules Presumably they were endogenously produced after the phagocytes accumulated the ferritin tracer within lysosomes In the present study since numerous lysosomes containing ferritin were found in control animals a normal turnover mechanism may be deduced However it cannot be totally dismissed that the select light cells in experimental animals also contain exogenously injected ferritin within lysosomes Finally it should be noted that although light cells often exhibited open-mouthed vesicles upon the basal lamina no ferritin labeling of these vesicles in experimental animals was found Certainly the scanty accumulation of ferritin within the basal lamina offered little probability of finding ferritin within such open-mouthed vesicles

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a few thin layers of the sheath or some loose layers of myelin were shown at some parts of the perikarya. The cytoplasm of the large unmyelinated neurons was rich with mitochondria in various forms curvature and length. Numerous short and elongated rough endoplasmic reticulum as well as ribosomes in rosette form were scattered throughout. Nissl bodies were randomly located and the Golgi network was not conspicuous. Round lysosomal granules were a common finding and lipofuscin masses were observed in specimens from individuals aged 7 years and older though their number and size did not increase in direct proportion to age. Intracellular neurofilaments were sparse. Toward the central process (axon) the cell organelles decreased and the neurofilaments and homogeneous substance increased. The axon hillocks were large and often long. Their processes became myelinated at a distance of about 4 to 38 μm from the perikarya. Only in one instance was a thick myelin formation observed near the axon hillock. Toward the peripheral process (dendrite) there was a decrease in cell organelles and there were considerably fewer neurofilaments than on the axonal side. The site of origin of the dendrite was often difficult to determine because of the small size. Unless the sections were examined at close intervals one would get the impression of a unipolar neuron or even a pathological specimen with atrophy of the dendrites. The dendritic process also became myelinated at a distance of 5 to 26 μm from the perikaryon (Fig. 2B) but myelination at the border of the perikaryon was not observed. The diameter of the dendritic process near the perikaryon was about one-fourth to three fourths the size of the axon. It came out straight or twisted, sometimes not directly opposite to the axon but close to it. As yet, no branching of these processes was seen at the proximity of the perikarya. No multipolar neurons were observed among the large neurons.

The large myelinated neurons were few in number (Fig. 3A B). Their size was similar to that of the unmyelinated type. They were located randomly within the neuronal masses throughout the cochlea and they were often seen in the aged individual. The highest count was made in a specimen from a 75-year-old 11 out of 39 or 28%. In the youngest individual (9 months) no myelinated neurons were found among the 94 neurons counted. The average of each individual percentage of specimens between the ages of 9 months and 42 years showed only 1% myelinated neurons. Between the ages of 65 and 92, the average increased to 19% excluding the Meniere's specimen. If the Meniere's case (age 59) which appeared to be normal, were included the average number of myelinated neurons became even smaller 5.8%. All cell counts were made from composite electron micrographs however the samples were mostly taken from the midportion of Rosenthal's canal as the bony parts along with some neurons had to be trimmed away for sectioning. The number of myelin layers varied somewhere in the order of 4 to 17 (Fig. 3B) and were often not uniform from one part to another part of the neuron. The neurons retaining a few sheaths of satellite cells or the partly myelinated types were classified as unmyelinated, since they would not be positively identifiable under low magnification or under the phase contrast microscope. Myelin sheath was observed in both loose and compact forms though the loose form was most common. The cytoplasmic characteristics of both myelinated and unmyelinated large neurons were similar.

The small spiral ganglion cell was 8 to 14 μm in diameter and 15 to 21 μm in length. There were both unmyelinated (Fig. 4A B) and myelinated (Fig. 5A B) types. Some small unmyelinated neurons found among the large neuron masses showed a cytological profile similar to the large ones. Another distinctly different type of small neuron was bipolar and a few cells might have been

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illaries and connective tissue as well as integrity of their neuronal processes environment, the function of the neurons I their fiber destinations may determine the ological characteristics of satellite cells or hwann cells

In the cat large unmyelinated neurons re not normally present and were only en in pathological specimens in which the yelinated type was converted into the unyelinated type (Spoendlin 1974) Although rge unmyelinated neurons were often obrved in the hydropic inner ear of the uinea pig (Kimura unpublished data) degenerative changes were not always associated with the loss of myelin sheath We bserved degenerated neurons with intact nyelin sheaths though the myelin was a oose form. Thus, it appears that neurons legenerate with or without the loss of myem sheath When we initially saw a majority of unmyelinated spiral ganglion cells in human Meniere's disease specimens we thought that these neurons might be one of the causes of the hearing loss (Kimura et al. 1976) However since all other human spiral ganglion cells from young and old individuals were unmyelinated such a notion was discarded

Spoendlin (1971 1972) suggested that the small unmyelinated neurons innervate the outer hair cells and Ross (1973) has suggested that the origin of these neurons is parasympathetic The population of these small unmyelinated neurons is small 5% in the cat (Spoendlin 1971) 10% in the guinea pig (Kellerhals et al. 1967) and 7 to 8% in the rat (Ross & Burkel 1973) Although our sample is rather small (due to our study being conducted at the ultrastructural level) the population was found to be about 6% The morphology of these small neurons differs somewhat among different species. For example in the cat they are unmyelinated and show either unipolar or bipolar processes Their cytoplasm is filamentous. The nuclei are lobulated eccentrically located, and the nucleolus is not very conspicuous. In the rat they are also unmyelinated but the cell processes are multipolar The cytoplasm contains abundant neurofilaments and cored vesicles the latter of which are thought to be one of the characteristics of parasympathetic neurons In our human material the small neurons are both myelinated and unmyelinated with either bipolar and/or multipolar processes The exact number of cell processes could not be determined due to the wide gaps or jumps from one section to the next in the area of particular interest. The cytoplasm was filamentous but dense-cored vesicles were not observed. The nuclei were round and more or less centrally located. Their nucleoli differed from those of the large neurons and showed loose and irregular forms. The only common morphological traits among the different species are that these neurons are small and the cytoplasm contains numerous neurofilaments Thus it is difficult to determine whether these neurons observed in each species correspond to each other functionally or whether there are other types of small neurons present which have not yet been identified From the present study it cannot be concluded whether these neurons innervate the organ of Corti or terminate somewhere in the modiolus or in the osseous spiral lamina. Nonetheless the neurons differ significantly in their cytoplasms from the majority of large neurons

Although it is not clear whether the function of small neurons is auditory or autonomic the large neurons forming the majority of the population are presumed to reach the organ of Corti based on studies by Retzius (1895) Held (1926) Lorente de Nó (1937) and Polyak et al (1946) When the nerve fibers were counted at the habenula and Corti's tunnel in the human at least 78 to 85% of the nerve fibers went to the inner hair cell area and a maximum of 15 to 22% of the fibers went toward the outer hair cells (Kimura, 1975) the percentages representing

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Grason-Stadler 1720 large errors would accrue.

Consequently the circuit shown diagrammatically in Fig. 1 was developed which performs a *vector* subtraction of the admittance of the ear canal. This is free of errors for all frequencies as long as the simple lumped-admittance representation of the ear canal is appropriate.

In the circuit, a signal at the probe tone frequency is derived from the probe tone oscillator and can be adjusted manually in magnitude and phase. The resultant signal is then subtracted from the AVC output signal by the differential amplifier shown in the figure. The magnitude and phase controls are adjusted with the ear canal pressure at 200 mm of water above the middle-ear pressure when the eardrum admittance is near zero to obtain zero output from the differential amplifier. Thus the ear canal admittance is cancelled out. Any subsequent measurements automatically give the admittance modulus at the eardrum based on the assumption that the eardrum admittance is zero when there is a static pressure differential across the eardrum of 200 mm of water.

The output of the differential amplifier is rectified and smoothed and there is provision to offset the output to remove any pedestal voltage and allow a high degree of subsequent amplification for sensitive recording of acoustic reflexes.

The circuit thus provides an output which is proportional to the admittance modulus at the eardrum and has a transient response which is essentially that of the AVC. The latter has been increased by a factor of two by halving a capacitance in the AVC circuit and Fig. ? illustrates the step response obtained by using a method similar to that of Popelka & Dubno (1978). Admittance modulus, conductance and susceptance outputs are compared in the figure. This illustrates that the admittance modulus output does not exhibit the overshoot which is apparent for conductance and susceptance outputs. The rise time of the admittance modulus is approximately 40 ms and this is adequate to record acoustic reflexes accurately in humans which Dallos (1964) has shown to have rise times greater than 120 ms.

Use of this circuit makes it possible to obtain acoustic reflex waveforms in terms of an absolute linear measure, the admittance modulus in the plane of the eardrum, at probe tone frequencies of 220 and 660 Hz effectively uncontaminated by the temporal response characteristics of the instrumentation. The accuracy of the apparatus has been extensively verified by comparing its steady-state output with the conductance and susceptance outputs and the complete admittance measurement system has proved to be reliable during four years of research work.

ZUSAMMENFASSUNG

Stapediusreflexmessungen, die mit dem Grason-Stadler 1720 Oto-Admittanzgerät durchgeführt werden, leiden unter einer meßgerätverursachten Überschwingung, die auf das Einschwingverhalten des Meßgerätes zurückzuführen ist. Um dieses Problem zu lösen, wurde eine besondere Schaltkreisanordnung entwickelt, die es ermöglicht, die zeitliche Charakteristik des Stapediusreflexes und ohne diesen meßgerätbedingten Fehler aufzuzeichnen.

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M. E. Lutman, Ph.D.
Inst. of Sound and Vibration Research
University of Southampton
Southampton
England

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J Santos Sacchi Ph.D
Center for Biomedical Education
City College Science Bldg Rm 915
The City University of New York
New York NY 10031
USA

Table II *Table illustrating the hair cell loss in animals belonging to groups A and B*

Injection of test solution was performed in the right ear. Hair cell loss below 1% was regarded as normal and was not noted in this table. In the cytocochleogram, counting of the most apical mm (18–19 mm from the round window) was excluded because of the great variation in hair cells present during normal conditions.
OHC outer hair cells, *IHC*, inner hair cells

nimal o	Hair cell loss (%) in various coils (right ear)							
	1st		2nd		3rd		4th	
	OHC	IHC	OHC	IHC	OHC	IHC	OHC	IHC
Group A								
							3	
					4 7		7.6	
•			12	5	29		8	5
Group B								
					6		7	

The left ear was normal with regard to hair cell count.

was performed as a double-blind testing model. After finishing the experiments the code for each solution was received and the various components were identified (Table I).

Each solution was administered only once into the right bulla tympanica via a fine needle through the tympanic membrane under an operating microscope after general anaesthesia of the animal using ether narcosis via an open mask.

One animal from each group was sacrificed for morphological analysis of the inner ear 24 hours post injectatem whilst the remaining animals were taken for morphology 5 weeks after the injection. The principles for morphological preparation of the membranous labyrinth (in situ immersion) have been described by Anniko & Lundquist (1979) using 1% osmic tetroxide as a fixative. All cochleae were prepared for cytocochleographic analysis, a technique described in detail by Ylikoski (1974), Spoendlin & Brun (1974) and others. According to the cytocochleographic study, selected parts were thereafter chosen for light microscopic sectioning, as also were all cristae ampullares and macula utriculi (staining: toluidine blue).

Five labyrinths from group A and six from group B were decalcified in a solution of EDTA (4.13%, pH 7.0–7.4) prior to further handling after fixation of the membranous labyrinth.

RESULTS

I *Clinical Observations*

A few minutes after injection with solution A or B the guinea pigs, already awake from the anaesthesia, revealed signs of marked vestibular dysfunction. Each animal showed a waltzing behaviour and nystagmus grade III with the fast component of eye movement towards *the right side*. These symptoms abated gradually after a couple of hours but could still be provoked 24–48 hours later. Guinea pigs injected with solution C remained normal.

On investigation 5 weeks post injectatem animals in groups A and B had either an impaired Preyer's reflex on the right side, or else this was completely lacking (most animals).

II *Middle Ear Cavity* (Figs. 1–4)

Solutions A and B

Animals investigated 24 hours after the intratympanic injection showed necrotic mucosal membranes and patchy haemorrhage in the middle ear.

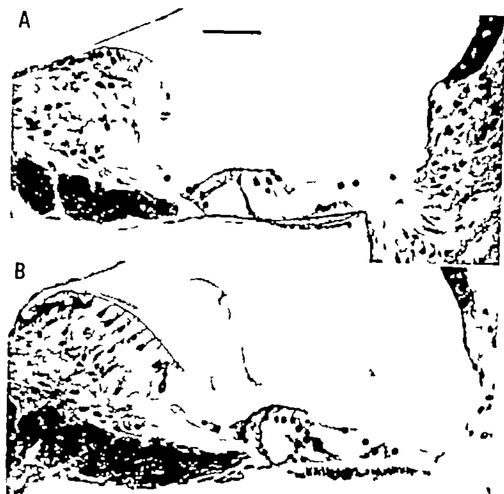


Fig 1 Two-micron thick araldite sections of the adult cochlea, stained with toluidine blue Bar represents 50 microns. (A) C57Bl untreated control. (B) C57Bl mouse which has been treated with thiouracil throughout its development. The tectorial membrane is grossly distorted

mutant genes are autosomal recessive but *Varitint Waddler* (Va) is autosomal semi dominant and heterozygous (Va/+) animals were used in this study All three groups of mice are deaf *Jerker* and *Varitint Waddler* mice also behavioural abnormalities and *Varitint Waddler* mutants have distinctive coat colours (Cloudman & Bunker 1945 Grüneberg et al 1941 Deol & Kocher 1958)

The mice were killed approximately two months after birth their otic capsules were removed and the cochleas cleared of sur rounding tissue and placed in fresh distilled water The tectorial membrane was separated from the organ of Corti and a finely drawn out glass pipette was used to transfer the pieces of membrane to several drops of fresh distilled water in turn until they were thor oughly rinsed and free of debris The pieces were then examined under phase contrast and stored at −20°C until required Each sample was collected either from the two cochleas of one animal or from four cochleas of two litter mates

The time taken between killing the animal and freezing the specimen was noted in each case and varied between 40 minutes and two hours This was not likely to lead to tissue damage as the tectorial membrane is an acellular matrix containing no lysosomes However in order to test this possiblity two samples were left at room temperature for 6 and 24 hours respectively before treatment. No significant differences were found in the banding pattern or intensity of staining of gels between these samples and those frozen only 40 minutes after death

As the tectorial membrane is normally insoluble sodium dodecyl sulphate (SDS) was used to dissociate the material into its protein subunits before subjecting the sample to elec trophoresis on disc polyacrylamide gels The

When the bulla tympanica was explored after 5 weeks the middle ear in most animals was extensively filled with superimposed newly formed bone. The ossicles and the cochlea were encapsulated by this bone (Figs. 3 & 4). On dissection, however, the ossicles could be freed fairly easily, in contrast to the cochlear bone wall which was in continuous contact with the newly formed bone.

When dividing the auditory and vestibular parts of the inner ear the gross structure of the membranous labyrinth was unaffected and no growth of bone or fibrous tissue was identified in the vestibulum immediately inside the stapedial footplate. The round window area in most cases (9/14) was covered with superimposed newly formed bone.

Solution B

The bulla tympanica was completely normal.

III. *The Inner Ear*

Solution A

The animal investigated 24 hours after the injection revealed a normal hair cell (HC) pattern at all levels in the cochlea, i.e. less than 1% HC loss, as also did 5/9 guinea pigs analysed 5 weeks after the injection. One animal died before morphological investigation was performed. Three remaining guinea pigs revealed a low percentage of HC loss (outer and inner hair cells, OHC and IHC, respectively) mainly in the apical coils (Table II).

Fig. 1. Left middle ear cavity from animal no. 9 in group A showing normal conditions (non injected side). The ossicles are removed. *Arrow*: round window membrane. Cf. Fig. 4.

Fig. Right cochlea from animal no. 2 (injected side) in group C showing normal gross morphology. A part of the bone covering the different coils has been removed. *Arrow*: round window.

Fig. 3. Right bulla tympanica from guinea pig no. 4 in group B. Extensive formation of new bone in the middle ear cavity. A part of the bone covering the cochlea has been removed.

Fig. 4. Right middle ear cavity from animal no. 9 in group A. Cf. Fig. 1. The cochlear wall is thickened due to superimposed bone. The ossicles cannot be identified. *Arrow*: indicating the round window region.

Light microscopic sections through the vestibular organs showed normal morphology in all but one animal (no. 8). All three cristae ampullares of this animal (on the injected side) showed degenerating hair cells of both types on the top of the cristae.

Solution B

In this group of guinea pigs all but one (no. 3) showed a normal morphology in the cochleae and cristae ampullares. The aberrant animal had a loss of 7% OHC in the 4th coil and 6% OHC missing in the 3rd turn, but an otherwise normal hair cell count (Table II).

Solution C

All 10 animals revealed a normal morphology in the inner ear organs.

COMMENT

It is difficult to establish an ototoxic effect in the present study. No signs of acute damage to cochlear hair cells and those of the vestibular organs occurred except in one animal. In the cochlea degenerated hair cells had been replaced by phalangeal scarring.

In only one guinea pig was there an obvious increased hair cell loss, indicative of a possible toxic effect of the drug, since the inner ear on the non-injected side showed normal conditions. The other animals with an outer hair cell loss below 10% in the apical turns might—according to the literature—be within the normal range of variation (Federspiel 1972, Ulehlová, 1973, Anniko & Sarkady 1978, Ulehlová & Voldrich 1978). The present control group of guinea pigs showed, however, less than 1% outer hair cell degeneration throughout the cochlea (except for the most distal part, 18–19 mm from the round window) thereby indicative of an ototoxic effect of the solutions A and B.

Whether or not the hair cell loss represents true ototoxic damage or is connected with or dependent upon the bone reaction in the bulla tympanica, e.g. disturbed microcirculation

parison of relative mobilities (Weber & Osborn 1969) The proteins used were RNA polymerase (165 000 155 000 39 000) β-galactosidase (135 000) phosphorylase a (92 500) bovine serum albumin (69 000) pyruvate kinase (60 000) catalase (57 500) phosphoglycerate kinase (47 000) aldolase (40 000) and glyceraldehyde 3-phosphate dehydrogenase (37 000)

Celloidin sections of cochleas from mice of each type were prepared by standard methods and these confirmed that the histological appearance of the tectorial membranes used in the present series was as described in previous reports (Deol 1954 1973 Deol & Kocher 1958)

An additional five samples of normal membranes from C57Bl mice were subjected to electrophoresis fixed and stained by the PAS technique (Fairbanks et al 1971) These gels were later stained with Coomassie blue to confirm the presence of the tectorial membrane proteins Gels containing known amounts of transferrin ovalbumin or ovomucoid were also stained using the PAS procedure to serve as controls

RESULTS

Isolated tectorial membranes from normal animals were virtually transparent until they were examined under phase contrast and some of the pieces became highly coiled in the distilled water As they were drawn through the narrow pipette tip the membranes were deformed but they seemed to be springy and quickly returned to their original shape when released

Membranes from thiouracil treated mice were generally more opaque with little tendency to form tight coils as in normal membranes When they were deformed by being constricted in a pipette they took a long time to return to their original shape and some showed no signs of returning at all within the duration of the dissection Their behaviour was more plastic than elastic and some behaved almost like formalin-fixed membranes

Isolated membranes from the three mutants appeared to be indistinguishable from normal tectorial membranes

The banding pattern of normal tectorial membranes from the four different genetic backgrounds is shown in Fig 2*a* The major band had a molecular weight of around 140 000 There were at least 3 components of higher molecular weight two sharp bands at 155 000 and 165 000 and a more diffuse band at 180 000 Further towards the anode a pair of bands at 105 000 and 95 000 could usually be identified and between 62 000 and 77 000 there was a wide area of staining which could often be resolved into at least five different bands. The bands close to the dye front were rather variable as can be seen in Fig. 2*a* Up to 2[illegible] bands could be distinguished by eye in any one gel

No significant differences could be detected in the banding pattern between abnormal and control tectorial membranes as shown in Fig 2*b*

The transferrin ovalbumin and ovomucoid bands stained bright pink in gels stained by the PAS method Ovomucoid contains 25% carbohydrate (Pearse 1968) so as about 20 μg of this glycoprotein was loaded there should have been around 5 μg of carbohydrate present Transferrin contains 6% carbohydrate but only 4% is Schiff positive (Simpson et al 1974) Again 20 μg was loaded so the staining was readily detectable when only 0 8 μg of reactive carbohydrate was present Ovalbumin contains only 4% carbohydrate and part of this is resistant to periodate oxidation (Eckhardt et al 1976) so a positive reaction was found in this case with less than 0 8 μg of suitable carbohydrate

No trace of staining was found for the tectorial membrane samples although the usual protein bands were observed when the gel was stained with Coomassie blue The amount of material in these samples as judged by the intensity of Coomassie blue staining was probably of the order of 5–10 μg per sample collected from two animals Assuming a value of

Acta Otolaryngol 89: 71–75 1980

ARTIFACTS IN EIGHTH CRANIAL NERVE BIOPSY

Y Collan J Ylikoski T Palva and R Selamaa

From the Departments of Pathology and Otolaryngology University of Helsinki Finland

(Received April 19 1978)

Abstract The occurrence of artifactual morphological changes was investigated in human eighth nerve biopsies and corresponding changes were brought about in biopsies from rats by mechanical handling before fixation. At the very site of compression by a pair of forceps the biopsy stained lighter than in its immediate surroundings. Electron microscopy showed denuded and fragmented axons among vesicular debris in the area. Further away the myelin coats were greatly thickened, often to the degree that the axonal canal could no longer be identified. The myelin lamellae showed separation and were occasionally seen in convoluted forms Also variations in the diameters of the nerve fibres and ruptures of the myelin coats could be produced after mechanical handling

Great caution should be taken to avoid artifacts in biopsies of nerve tissue This is a good general rule accepted by most research workers in the field of neuropathology It is quite obvious however that this cannot always be avoided. In experimental work perfusion fixation gives superb preservation but biopsies from man may be subject to artifacts because of handling or autolysis before fixation. Under certain circumstances the available biopsies are necessarily very small and in these artifactual changes are most imminent. Our project to study the neuropathology of the human eigth nerve and the inner ear (Ylikoski et al. 1978*a* Ylikoski et al 1978*b* Palva et al 1978) included a series of experiments relative to artifacts which may arise in these structures as a result of handling. The findings are reported in this study

MATERIAL AND METHODS

For this study 150 biopsies of eighth nerve were available. The biopsies were carefully checked for sites of compression caused by a pair of forceps Sites of compression were studied by light and electron microscopy In two cases the biopsy was cut in two and one half was artificially crushed several times with small forceps in order to create numerous artifacts These samples were compared with the other biopsies In addition three 1 year-old male rats were anaesthetized with a barbiturate preparation and the eighth and fifth nerve uncovered by the occipital route Biopsies were taken on the right side with a biopsy forceps whereupon the whole body of the animal was perfused with 3% glutaraldehyde in 1% phosphate buffer through a cannula inserted into the aorta. The fixative was allowed to act for 15 min whereafter biopsies were taken from the left side The biopsies were fixed further in the same fixative at +4°C for 24 h and thereafter transferred into sucrose They were postfixed in 1% OsO in 1% phosphate buffer and embedded in Epon Sections were cut with glass knives for light and electron microscopy

RESULTS

In light microscopy several sites with doubtless compression artifacts were found in human biopsies (Figs 1–3) At the very site of compression the biopsy appeared lighter than in the surrounding zone that was composed of nerve fibres in which the axon was totally or

This study was supported by a grant from Signe and Ane Gyllenberg Foundation.

brane by treatment with SDS Free carbohydrate which is not bound to SDS or to an SDS-protein complex would not enter the gel during electrophoresis as it would not have a sufficiently high charge to mass ratio and so it would remain undetected even though it might be present in the sample

ACKNOWLEDGEMENTS

I would like to thank Dr M S Deol and Dr Yvonne Edwards for their advice and encouragement Mrs Diane Kyriakou for her help in looking after the mice and Dr Ian Bock for translating the summary This work was supported in part by an M R.C. Studentship

ZUSAMMENFASSUNG

Die Proteine der normalen Membrana tectoria der Maus wurden durch SDS Polyacrylamid-Gel-Elektrophorese analysiert Drei wichtige Proteinbänder mit Molekulargewichten von 145 000 155 000 und 165 000 und eine Anzahl kleinerer Teile wurden gefunden Abnorme Membrana tectoria von Mäusen die man mit Thiouracil behandelte und von *Deafness Jerke* und *Varitint Waddler* Mutanten wurden auch in dieser Weise geprüft aber es gab keinen Beweis die *Hypothese zu bevorzugen, daß eine* abnorme Proteinbeschaffenheit die Verzerrung der Membranen hätte verursachen können. Kein PAS-positives Material ließ *sich in Gelen der normalen Membranen* demonstrieren

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K P St el Ph.D
MRC Institute of Hearing Research
U iver ty of Nottingham
Nott gham NG7 2UH
England



Fig. 4 Peripheral parts of biopsy taken from the vestibular nerve of normal male rat with a biopsy forceps before perfusion fixation. Note thickening of the myelin in many fibres and replacement of the axoplasm by myelin material. 687

Fig. 5 A biopsy of a rat vestibular nerve taken before perfusion fixation. There are numerous changes that in most cases should be considered artifacts. At several places there is evidence of rupture of the myelin layer widening of the nerve fibres and myelin material replacing parts of the axoplasm inside the myelin coat. Artifactual tearing of capillaries has also occurred and erythrocytes are seen between the nerve fibres. 713

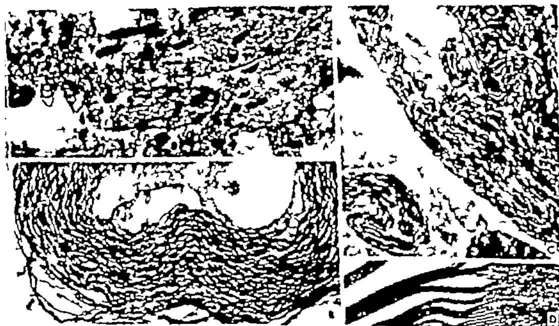


Fig. 6 (A) Denuded axons in an area which has been pinched with biopsy forceps. Around the axons there is vesicular debris left by the myelin coats and the Schwann cells surrounding the fibres. Biopsy taken before perfusion fixation from the 8th nerve of rat. 9600. (B) Greatly thickened myelin coat of nerve fibre in an area of compression in 8th nerve biopsy from laboratory rat. There are two large adaxonal vacuoles between the myelin coat and the axon. 7900. (C) Human 8th nerve biopsy. Greatly thickened myelin layer with convoluted groups of lamellae which also show separation in places. The axon is compressed at the periphery of the fibre. The greatly thickened myelin layers with convoluted myelin lamellae were more common in samples showing evidence of compression than in samples which did not. 8400. (D) Separation of myelin lamellae at the minor dense lines. 78000

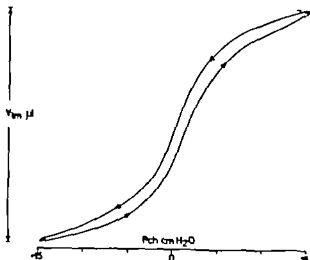


Fig 1 Recording of the volume/pressure curve of the tympanic membrane system. The recording shows how the volume/pressure curve follows different tracks when the pressure decreases and increases—hysteresis.

using a pressure chamber and the method worked out by Ingelstedt et al (1967) and Elner et al (1971*a*) The main principle of the method is to record the volume deviation of the tympanic membrane in relation to its neutral position at changes of ambient as well as of middle ear pressure The method also makes it possible to study the elastic properties of the tympanic membrane system recorded as a volume/pressure relationship This quotient is defined as the volume deviation of the eardrum Vtm in relation to its neutral position and as a function of pressure differences across the eardrum Vtm= f(Ptm) The method has been described in detail by Elner et al (1971*c*)

In the present study the volume/pressure relationship was recorded within a pressure range of ±15 cmH_2O The Eustachian tubes were closed during the recordings and the chamber pressure was kept constant at +15 and −15 cmH_2O for 3 sec (Fig. 1) The total volume displacement of the tympanic membrane Vtm was calculated between these static pressure points This pressure range 30 cmH_2O was the same as that used by Elner et al (1971*c*)

The material was divided into four different tubal function groups according to Elner et al 1971*b*)

Group I equilibrates pressure difference across the eardrum completely

Group II equilibrates pressure difference with small residual pressure in the middle ear

Group III equilibrates a relative overpressure but not an underpressure

Group IV is incapable of equilibrating over and underpressure by deglutitions

Group I was divided into subgroups

Ia patulous tube

Ib equilibrates over and underpressure of 10 cmH_2O in the middle ear by 1–3 deglutitions

Ic equilibrates over and underpressure of 10 cmH_2O in the middle ear by 4–10 deglutitions

MATERIAL

The material consisted of 24 experienced scuba sport divers (46 ears) 26–34 years old (mean 29) with normal ear findings and without any signs of catarrhal infection on the day of the experiments They had been diving for about 5 years with a mean of 350 logged dives. Twenty divers had a normal hearing within 0–20 dB (related to ISO standard 1964) Four divers had a sensorineural hearing loss The hearing loss was known since early youth in two of the divers The results were compared with the results obtained from 98 subjects (98

Table I *The distribution in different tubal function groups*

Tubal function groups	Divers No of ears	Divers %	Non-divers No of ears	Non-divers %
Ib	18	40	33	34
Ic	12	77	37	38
II	11	4	21	1
III IV	4	9	7	7
Total	45	100	98	100

vesicular degeneration of the myelin found in spinal cord trauma, corresponds to the separation of myelin lamellae in the thickened myelin coats in our study and appears to be initiated by mechanical damage. The full development of this change and the development of vacuoles inside myelin appears to be due to active changes in fluid compartments of the nerve fibres. We did not observe such a change probably because the samples were fixed immediately after biopsy.

ZUSAMMENFASSUNG

Das Vorkommen von artefaktuellen Veränderungen in Biopsien vom 8. Hirnnerv des Menschen wurde untersucht und entsprechende Veränderungen wurden in Biopsien von Ratten durch mechanische Behandlung vor der Fixation verursacht. Die Färbung der Nervenfasern war schwächer an der Stelle der Kompression als in der Umgebung. In elektronenmikroskopischen Untersuchungen beobachtete man bare fragmentierte Axone und vesikuläres Debris. Ferner in der Umgebung beobachtete man verdickte Markscheiden und in vielen Fällen konnte man den Axon nicht mehr sehen. Die Myelinlamellen waren teilweise separiert und oft in rundliche Formationen reorganisiert. Variationen in Diametern der Nerven und Rupture der Markscheiden wurden auch nach der mechanischen Behandlung vor der chemischen Fixation beobachtet.

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Yrjö Collan, M.D.
Department of Pathology
University of Helsinki
Finland

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The volume/pressure quotient of the tympanic membrane system in divers had a higher value than that in non-divers This difference is most probably due to the frequently large pressure load during diving stretching the tympanic membrane system The often rapid increase in ambient pressure during descent makes it necessary for the diver to equilibrate by means of Valsalva s manoeuvre and this will induce large changes in the middle ear pressure from negative to positive In subjects without diving experience Elner et al (1971 *c*) found a correlation between the mobility of the tympanic membrane system and the capacity for middle ear equilibration i e Eustachian tubal function increased mobility was found with a reduced tubal function Such a correlation was also seen in divers in the present study

The mobility of the tympanic membrane system in divers belonging to groups I*b* and I*c* was significantly higher than in non-divers of the same tubal function groups while no significant difference was seen between divers and non-divers belonging to groups II III and IV These results were surprising as it might have been expected that divers with a less good tubal function would have their tympanic membrane system exposed more often and to greater pressure differences during diving. The absence of difference in these groups might be explained by a distension limit of the elastic elements of the tympanic membrane system

ZUSAMMENFASSUNG

Das Verhältnis zwischen Volumen und Druck im System des Trommelfells wurde bei 4 Sporttauchern untersucht *Die Befunde wurde mit Befunden* von 98 Nichttauchern verglichen. Unter den Personen mit guter Tubenfunktion hatten die Taucher im Vergleich zu den Nichttauchern eine vermehrte Beweglichkeit des Trommelfellsystems Taucher mit reduzierter Tubenfunktion unterschieden sich nicht von *Nichttauchern mit reduzierter* Tubenfunktion Faktoren die das Verhältnis zwischen Volumen und Druck im System des Trommelfells beeinflussen, werden diskutiert

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Orjan Tjernström M.D
University of Lund ENT Departm nt
Malmo General Hospit l
S 21401 Malmo S ed n



Fig. 3 Detail of Fig. 3. The organ of Corti and the ganglion cells are absent.

unreliable. This check was made on both sides. The destruction was considered complete when no microphonic or action potentials higher than 5 μV could be measured. A fenestrum was made in the lateral canal on one side near the ampulla. An electrode was placed on the edge of the fenestrum and when microphonic and AP's were detectable an equal response curve for 10 μV was recorded for the microphonic.

In the week after the fenestration the nystagmogram and the Tullio reaction were checked and the animal was again conditioned to sound. Then, after about a week, we tried to obtain a behavioural response to sound by means of a shiver audiogram.

We observed the animals for about ? months after fenestration. At the end of the program the animals were sacrificed and prepared for microscopic examination.

Experiments with pigeons

From seven 1 year-old pigeons of about 600 g the cochlea was removed. In contrast to the guinea pig this can easily be done in a pigeon without destroying the vestibular organ (Bleeker & de Vries 1949; van Eyck, 1955). The same anaesthesia with oxygen and fluothane was used. The skull was opened in the anterior inferior part of the cross formed by the posterior and lateral canal (Ewald, 1892). After removal of the external plate of the skull the recessus scalae tympani, the columella, the round window and the labyrinthine canals

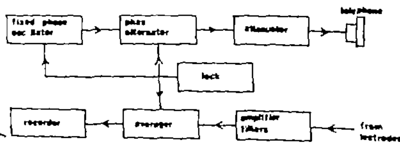


Fig. 4 Apparatus for measurement of microphonic and action potentials from pigeons.

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Orjan Tjernström M.D
University of Lund ENT Department
Malmo General Hospital
S 214 01 Malmo Sweden

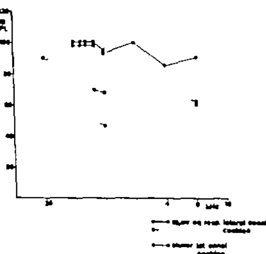


Fig. 7. Guinea pig 15. Equal response curves of the microphonics and shiver thresholds obtained from the intact cochlea and from the lateral canal after bilateral destruction of the cochlea and fenestration of the lateral canal.

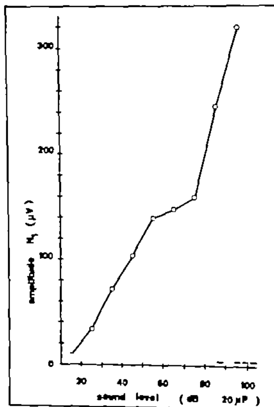


Fig. 8. Amplitude of compound action potential (N_1) before destruction of the cochlea. The amplitude was below the dotted line (5 µV level) after destruction of the cochlea.

cedure took much time because three operations on each animal were necessary. This created many opportunities for failure. Some animals died during the procedure, others developed otitis. Sometimes the laser radiation was too intensive, causing damage to the labyrinthine structures. In other cases the radiation was too weak, causing incomplete destruction of the cochlea and a rest effect of cochlear microphonic and action potentials. Of the 20 animals, only two showed a significant and clear behavioural response together with the electrical responses after fenestration, a response not obtainable before fenestration and after destruction of both cochleae.

Figs. 6 and 7 show the results in one of the animals (no. 15). The fact that the shiver audiogram showed behavioural response for frequencies as high as 8 kHz, whereas the fact that microphonics above 1 kHz were not obtainable can possibly be explained by supposing that the animal reacted at the onset of the acoustic stimulus. The second animal (no. 18) showed nearly identical curves. Fig. 8 gives the input–output curve for the compound action potential for one of the guinea pigs measured before destruction of the cochlea (the other animals gave similar results). In despite of the simplicity of the apparatus used to measure these action potentials a large degree of malfunction of the cochlea after laser radiation could be proven with it.

Pigeon experiments

In the second program, electrophysiological results were obtained from 7 pigeons. The results of the microphonic measurements are given in Figs. 9 and 10. The latter figure shows clearly that microphonics have almost disappeared after extirpation of the cochlea. After fenestration, much larger microphonic-like signals could again be detected. Before extirpation of the cochlea, action potentials could be obtained with high as well as with lower stimulus frequencies (Fig. 11). After ex

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Orjan Tjernstrom M.D
University of Lund ENT Department
Malmo General Ho pital
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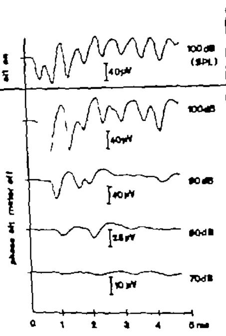


Fig 12 Action potentials from pigeon after cochlear extirpation and fenestration The upper part of the figure as obtained with 1000 Hz tone burst (4 ms duration) delivered to the telephone with alternating phase. For the lower part of the figure the phase alternator was switched off

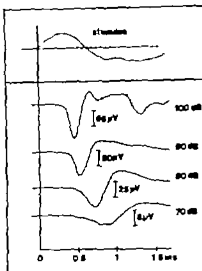


Fig 13 Action potentials from a pigeon after cochlear extirpation and fenestration. The sound pressure of the stimulus is given in the upper part of the figure (same time scale as lower part) Stimulus level is given as peak equivalent sound pressure level.

levels Before cochlea exturpation the action potential latency ranges from 0 9 to 2 ms

CONCLUSION

This work is a preliminary report With these few results we can only indicate that in guinea pigs and pigeons with total cochlear loss the labyrinth might perhaps give some meaningful sonic information after fenestration of the lateral canal.

On account of the simplicity of the operation, fenestration of the lateral canal in patients with total cochlear loss on both sides can be considered as an alternative to monopolar cochlear implants. The middle ear can be left intact. There is no need to create a cavity as in the original fenestration operation for otosclerosis. Caloric vestibular disturbances are therefore of minor importance Closure of the round window to ameliorate the efficiency of the fenestration might be considered We can only guess about the meaning of this vestibular signal obtained by sound stimuli. It can hardly be expected that frequency discrimination is possible—apart perhaps from very low frequencies But the quality of the information might be equal to that obtained by monopolar cochlear implants

ZUSAMMENFASSUNG

In einem Bericht von Untersuchungen an Meerschweinchen und Tauben werden die elektrophysiologischen Resultate und das Reflexverhalten von akustischer Reizung des Labyrinthes gemeldet. Beim Meerschweinchen waren beiderseits die Schnecke ausgeschaltet mittels Laserstrahlung und der laterale Kanal fenestriert. Die Schnecke der Tauben wurde vor der Fenestration entfernt. Auf Grund der Resultate wird darauf hingewiesen daß möglicherweise bei Patienten mit totalem Ausfall des Gehörs beiderseits mittels einer Fenestration des lateralen Kanales ähnliche Resultate erreicht werden können als mit monopolaren „cochlear implants"

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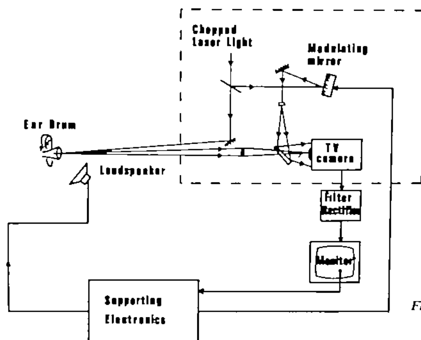


Fig 1 ESPI design

can be simply said to represent the object s position relative to a reference point measured in terms of the wavelength of the laserlight If the object changes its position during the hologram exposure the resulting change in phase relationship is recorded by the holographic process When afterwards the reconstructed object is seen through the hologram the object is covered with a pattern of dark and bright fringes This fringe pattern can be used like contours on an ordinary map to determine the object s movement When holography is used for such measuring purposes it is called holographic interferometry Holographic interferometry is a very sensitive measuring technique as the wavelength of light is used as the unit

Tonndorf & Khanna (1972) used holographic vibration analysis in investigating the movements of the drum in the cat Gundersen & Høgmoen (1976) used the same method in vibration measurements on human temporal bone preparations

However the holographic process is slow and complicated because it is based upon exposure and development of high resolution filmplates

If the filmregistration process is replaced by TV camera recording with subsequent electronic filtering and display on a TV monitor we gain immensely in speed and ease of handling Exposure time is short 1/25 sec and a new hologram is also created every 1/25 sec The TV holography system which is usually called electronic speckle pattern interferometry or ESPI is very insensitive towards objects instability and external vibrations It is therefore well suited for work on unstable objects like ear preparations (Høgmoen & Løkberg 1977)

The background for this investigation is our work on increasing the application range of ESPI for vibration analysis mainly based on reference wave modulation techniques Recently we have applied ESPI to measure the vibratory motion of the eardrum in living man

METHOD

Fig 1 gives a schematic presentation of our ESPI design ESPI is a holographic method which uses the target of a TV camera as the registration medium for the interference patterns The hologram reconstruction is performed electronically by filtering and rectifying the videosignal from the camera and the resulting interferograms are presented in real-

Acta Otolaryngol 89: 85–92, 1980

ANALYSIS OF THE MECHANICAL IMPEDANCE OF BONE ANCHORED HEARING AIDS

A Tjellström B Håkansson[2] J Lindström P I Brånemark, O Hallén U Rosenhall and A Leyon[4]

From ENT-Department Sahlgren Hospital, University of Göteborg Göteborg [2]Laboratory of Medical Electronics Department of Applied Electronics Chalmers University of Technology Göteborg [3]Institute for Applied Biotechnology Mölndal and [4]Department of Audiology Sahlgren Hospital, University of Göteborg Göteborg Sweden

(Received April 2, 1979)

Abstract Some patients who need hearing aids are unable to use an apparatus which transmit the sound via the external ear canal and have to use a bone conduction hearing aid The bone vibration transducer of this aid is applied to the skin over the mastoid process and the sound is transmitted via the soft tissue and bone to the cochlea The pressure needed to apply the transducer often gives the patient discomfort and the damping effect of the soft tissue gives poor quality of the sound transmitted Advances in the ability to permanently implant foreign material in the body and perform permanent skin penetration has made it possible to develop bone-anchored hearing aid Fourteen patients have been equipped with such hearing aids T be able to give these patients the best hearing aid, new transducer has to be constructed to match the new situation The impedance of the bone anchored titaniumscrew/skull has been studied and the resistance and reactance of the mechanical impedance have been measured The influence of damping soft tissue layer over the bone has been analyzed The difference between the impedance of the skull and the impedance of the soft tissue + skull was in the order of 10 to 25 dB depending on the frequency

For various reasons some patients who need hearing aids cannot use an apparatus in which the sound is transmitted via a plastic ear mould in the external auditory canal but require a bone conduction hearing aid instead This means that the sound is transmitted to a bone conduction receiver applied to the skin over the mastoid process by means of a steel spring over the head via soft tissue and bone into the cochlea. One reason why the patients must use such a hearing aid can be a chronic ear condition and that the ear starts to discharge as soon as the auditory canal is blocked by an ear mould Other reasons are extreme transmission impairments such as gross malformation of the ear canal and the middle ear and also extensive otosclerosis

Patients with bone conduction hearing aids often complain about the pressure with which the hearing aid must be applied This pressure is so great that it causes pain and one often sees reddening and irritated skin areas which indicate where the hearing aid has compressed the skin. The position of the apparatus must often be adjusted so that the patient can get the best hearing This may be due to the roughness and convex nature of the bone surface under the skin which also hinders an exact and stable position of the hearing aid Many patients complain about the poor sound quality which characterizes this type of hearing aid Furhter the steel spring over the head for holding the hearing aid in position can give esthetic problems for thin-haired men as well

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This paper is the first in series of presentations concerning an inter-disciplinary development program on bone-anchored hearing aids. The work is done in close cooperation between the Department of Otolaryngology University of Göteborg (Head Prof. Olle Hallén) The Institute for Applied Biotechnology Mölndal (Head Prof P-I Brånemark), The Department of Audiology University of Göteborg (Head Prof. G Lidén) and the Laboratory of Medical Electronics, Department of Applied Electronics, Chalmers University of Technology Goteborg (Head Prof R Magnusson)

This study was supported by grant from Stiftelsen Tysta Skolan, Stockholm, The Swedish Medical Research Council, project No B 79-17X-05436-01 and The Goteborg Medical Society Goteborg

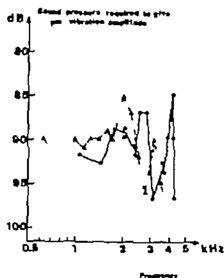


Fig 3 Frequency response of the malleus manubrium tip of male subjects. (Sound pressure level given in dB re 2–10⁻⁵ N/m)

lated linearly as indicated which eased the alignment procedure considerably In addition the subject could watch the tympanic membrane interferograms on a separate TV monitor which enhanced advantageous co-operation

The ear tube was painted dull black on the inside but no precautions such as spraying with bronze powder were taken to improve the optical properties of the membrane itself The optical properties of the object are difficult, with variations in reflectivity across the membrane and with a large component of internally reflected light because it is semi transparent This would ordinarily result in very low contrast in the interference patterns But by modulating the reference wave with an amplitude that corresponded to a zero point of the time average characteristic fringe function (the 1st and 2nd zero point were used) this problem was solved since the unwanted light normally coincided with zero intensity Light reflected from parts of the membrane that vibrated with the same amplitude and phase as the reference mirror however would coincide with maximum intensity

The eardrum was stimulated by the free sound field from a loudspeaker The sound pressure level (SPL) was monitored by a microphone positioned close to the ea SPL values cited in this paper must b with care as they varied quite consid with the position of the microphone future work the SPL should be measu near the drum as possible to give reprod results

During the experiments extensive us made of a videotape recorder for subse analysis of and measurements on the rec interferograms However the real-tim ture of the ESPI was absolutely necessa all the adjustments of different param such as reference wave modulation obje citation and ESPI parameters which had performed during the experiments

Photoelectric measurements from the recordings were made to obtain absolut plitude values at low sound pressures su 70–80 dB with amplitudes in the range of l and below Phase variations across the r brane were also measured

RESULTS

The preliminary experiments have been dertaken to demonstrate that ESPI w continuous laser can be used to measure drum vibration on living objects The ex ments have also been performed under improvised conditions which do not inv large series of measurements

Fig. 3 shows the frequency response c of the malleus manubrium tip of the left e two male subjects It gives the SPL neces to obtain the first dark fringe (correspon to 0 1 μm amplitude) as a function of quency The shape of the curve is simila the curves Gundersen (1971) observed u an electromagnetic pick-up

Both subjects showed a point of resona in the region of 2 kHz. In temporal bone p parations the point of resonance was betw 800 and 1200 Hz The shifting of the point resonance to higher frequencies in living m may be due to contractions of the musc of the middle ear

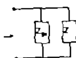


Fig 2 Input impedance to the head (Z) where Z_{st} corresponds to the impedance of skin and subcutaneous tissue and Z_s corresponds to the impedance of the skull

months and no fixture has loosened from the bone (February 1979) Depending on the immature technical design a couple of the patients have complained about minor inconvenience from the fixtures The hearing aids used by these patients have been of the same manufacture and the same type as their usual hearing aids

The bone-anchored hearing aids operates with a changed input impedance looking into the head The input impedance together with a given hearing aid with its associated electronics determines the input power to the head The variation of the power with frequency is different for bone-anchored transmission and conventional skin and bone transmission. The patients used the same manufacture and type of hearing aids as before and most of them feel that the sound has a better quality for bone-anchored transmission than for conventional bone transmission Optimization of the hearing aid should lead to a further increase in the sound quality Such optimization requires a closer study of the impedance in the system Psychoacoustic analysis must be carried out in order first to further develop the hearing aid and later to match the associated electronics.

The purpose of the study present here is to describe and investigate the mechanical system when an osseointegrated entrance to the skull is used. A comparison with the ISO/IEC standard for mechanical impedance in skin-head will also be carried out (Flottorp /Solberg, 1976)

Models—electrical analogs

Mechanical systems, of which sound transmission is one, are complicated and can best be described by models These can be mechanical and can also be translated to electrical analogs The mass corresponds to an inductance (L) the elasticity to capacitance (C) and damping to resistance (R) The head with soft tissue and bone supported by soft tissue of the neck and neck bones can be considered a mechanical system. To study this system two general models have been proposed In one of the models shown in Fig 2, the impedance of the skin and subcutaneous tissue is discribed by Z_{st} In the other model shown in Fig. 3 the impedance of the snap fastener is described by Z_{sf} and Z describes the impedance of the bone of the skull The impedance of the osseointegrated titanium fixture is negligible because its mass is very small and the physical properties of titanium correspond well with those of bone The construction of the snap fastener is shown schematically in Fig 1 These two models describe the input impedance into the head in the frequency range 250 to 8 000 Hz, with and without skin penetration respectively If the mechanical snap fastener is stiffer than the skin including the subcutaneous tissue over the mastoid process the snap fastener system Z is better than the system with subcutaneous tissue Z_1

MEASUREMENT APPARATUS AND METHOD

The measurements were made with Bruel & Kjaer's impedance measurement apparatus consisting mainly of an impedance head and a vibrator A block diagram of the measurement apparatus is shown in Fig 4 The impedance of the mass in the driving platform in the impedance head has been taken into account.

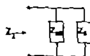


Fig 3 Input impedance to the head (Z_T) where Z_{sf} corresponds to the impedance of the snap fastener and Z_s corresponds to the impedance of the skull

rungszeit jedes Fernsehbildes erreicht. Die Amplituden und Phaseverteilung des Trommelfells wurden mittels der Phasemodellierungstechniken erfaßt, die auch die Schwingungen im Zeitlupentempo darstellen konnten Bei photoelektrischer Aufdeckung der Intensitat des Fernsehbildes konnten Amplituden bis zu nm gemessen werden Vorläufiger Erfolg wird präsentiert

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T Gundersen M D
Department of Ear Nose Throat
Regional Hospital
7000 Trondheim Norway

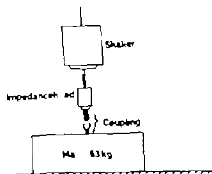


Fig 6 Measurement apparatus for impedance measurements on snap fastener only

RESULTS

The results of the magnitude (in mechanical ohms) and phase (in degrees) measurements are presented in Figs 7 and 8 The curves show an average of the measurement results in eight patients A scattering in the magnitude measurements of about ±30% is noted for frequencies below 1 250 Hz and over 5 000 Hz. Between these frequencies the scattering is less than ±12% The bump at 1 250 Hz is relatively flat in some cases The curves obtained for the various patients all had the same characteristics From the magnitude and phase curves the resistive and reactive impedance have been calculated and are shown in Fig. 9 The scattering of phase is larger than that of magnitude which is caused by greater indi-

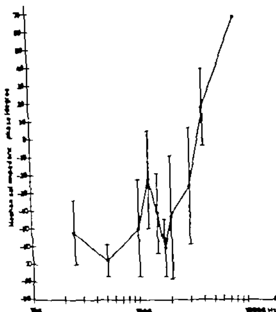


Fig 8 Phase of the mechanical impedance (average) The scatter of the 8 patients is shown

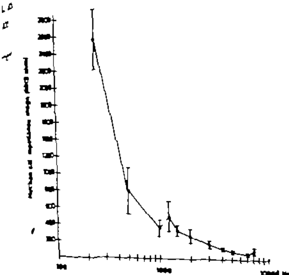


Fig 7 Magnitude of the mechanical impedance (average). The scatter of the 8 patients is shown

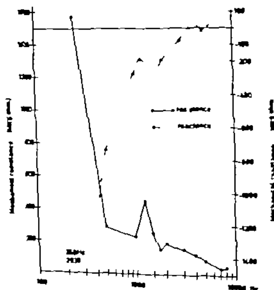


Fig 9 Resistance and reactance of the mechanical impedance

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T Gunderse M.D
Department of Ear Nose Throat
Regional Hospital
7000 Trondheim Norway

Acta Otolaryngol 89 93–104 1980

DEFECTS OF THE OTOCONIAL MEMBRANES IN NORMAL GUINEA PIGS

Lars-Goran Johnsson, Charles G Wright, Robert E Preston and Pamela J Henry

From the Kr ge Hearing Research Institute Department of Otorhinolaryngology University of Michigan Medical School Ann Arbor Michigan and from the Callier C nter for Communication Disorders The University of Texas at Dallas Dallas Texas, USA

(Received March 23 1979)

Abstract Otoconial membranes from large number of normal albino and pigmented guinea pigs were examined by light microscopy In more than 20% of the animals, microdissection revealed that the crystalline layer of the otoconial membrane was incomplete leaving large areas of the underlying gelatinous layer uncovered by crystals. These defects ere bilateral, more or less symmetrical, and invariably more extensive in the saccule than in the utricle In two animals, large single conglomerates of crystals were found on both the saccular and utricular maculae None of the animals displayed abnormal behavior or posture and no obvious cause for the otoconial defects as found. The presence of hereditary congenital condition is postulated The high incidence of otoconial defects in guinea pigs makes them poorly suited for most investigations of experimentally induced pathology Therefore previous reports of otoconial pathology in guinea pigs should be viewed in the light of these findings

The purpose of this paper is to report what appears to represent a marked reduction of saccular and utricular otoconia in a large number of untreated guinea pigs which were used as normal material in our laboratory This unexpected finding was made during dissection of the vestibular system in 168 guinea pigs for a study of the incorporation of ^{45}Ca in the otoconial membranes

The normal appearance of the otoconial membrane in the guinea pig has been described by several investigators who have used the technique of microdissection, which allows a direct inspection of the otoconia (Engström et al. 1966 Hawkins & Johnsson 1976) The most detailed description is given by Lindeman (1969) and the reader is referred to his monograph concerning the normal morphology of the vestibular apparatus The otoconial membrane in man has been described in adults (de Burlet & Hoffman 1929 Johnsson & Hawkins 1967) in infants (Wright & Hubbard 1978) and in fetuses (Wright Hubbard & Clark, 1979*a*) Scanning electron microscopy has given more precise information about the shape and structure of the otoconia in man (Ross et al 1976 Wright & Hubbard 1978) as well as in rodents (Kellerhals et al 1970 Lim, 1973*a b* Ross & Peacor 1975)

In most previous descriptions and illustrations of the normal macular organs the otoconial layer covers the entire surface of the neuro-epithelium The layer of otoconia is continuous appears snow white and is strikingly displayed The surface is uneven and the otoconia form a characteristic ridge or crest the so-called snowdrift as it is referred to by Engström et al. (1966) The curvatures of the snowdrift lines in man and guinea pigs are similar in shape In the saccule the line is gently curved while it is more horseshoe or U" shaped in the utricle (Fig. 1) The snowdrift covers approximately Werner's (1940) *striola* ('little stripe') i e the area of the neuro-epithelium containing the greatest concentration of Type I hair cells.

Little is known about the possible metabolism of the otoconia. Uptake of ^{45}Ca by the otoconial membranes in gerbils (Preston et al 1975) and in guinea pigs has been demonstrated The incorporation of ^{45}Ca can be altered by administration of ototoxic drugs (Mechigian et al. 1979) These findings suggest growth and neogenesis of otoconia. Lim

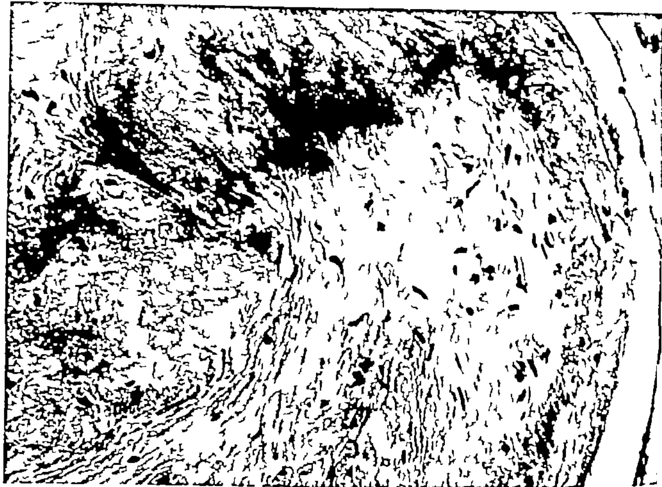


Fig 1 Tympanosclerosis with increase of collagenous fibres hyalinization and calcification (Haematoxylin-eosin ×135)

Rannie (1963) claims that there is less wrinkling and detachment of de-paraffinized sections

Electron microscopical preparation

Identical samples were placed in phosphate buffered 3% glutaraldehyde (pH=7 2) for 3 h at room temperature and post fixed in 1% buffered OsO_4 for 2 h at 4°C After post fixation the tissues were dehydrated through ethanols and propylene oxide and embedded in Epon 812 Sections were stained with uranyl acetate and examined in a Zeiss EM 9

RESULTS

Histology

The histological observations with the haematoxylin-eosin stain resembled the description of other investigators (Beck & Ebert 1964) increase of collagenous fibres hyalin degeneration and calcium deposits (Fig. 1)

The connective tissue was poor in cells The dominant cell type was fibroblasts The vascularization was sparse There was no sign of granulation tissue The overlying epithelium in some locations was intact in some locations there was a defect

Histochemistry

Material which reacted with aldehyde fuchsin but not Azan orcein or periodic acid Schiff sequence was seen in previously oxidized sections from all tympanosclerotic plaques (Fig 2*a b*) According to their histochemical definition (Fullmer et al 1974) these fibres are oxytalan fibres

In tympanosclerosis the fibre distribution

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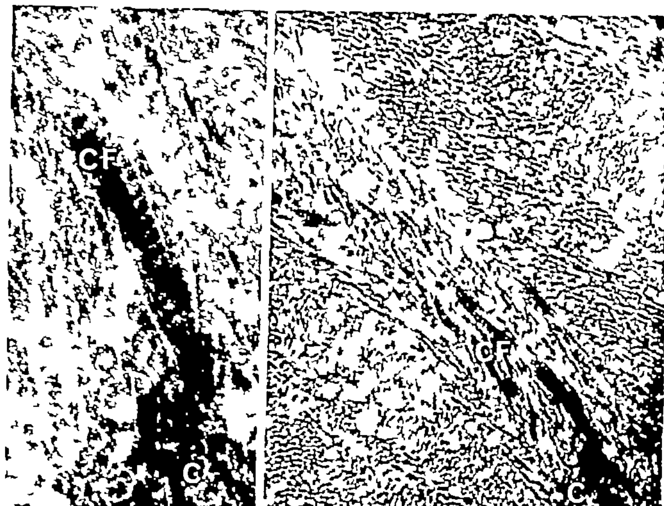


Fig 3a Abnormal large collagenous fibrils with atypical cross-linkages. *CF*=collagenous fibrils *C*=calcification (× 1120) *Inset* Higher magnification of the indicated area in fig 3*a* ( ) (×63 360)

was not uniform but showed rather focal aggregations There was multiple branching (Fig 2*c*) and related necrosis Oxytalan fibres were not seen in vicinity to blood vessels and were never observed in intact tissue

Electron microscopy

Extracellular space The numbers of collagenous fibres were increased In most cases their elements ran in various directions loosely or densely packed together

In addition foci of degraded collagen fibres appeared These elements were characterized by an increasing loss of the typical fibril periodicity and by fibril swelling An amorphous electron-dense material was often seen in the matrix It tended to be concentrated either about areas where collagen fibrils appeared to be swelling and losing their characteristic banding pattern or in areas where collagen fibrils were fragmented and appeared to be undergoing dissolution (Fig 3*a* *b*)

In close vicinity to the borders of the collagen fibrillar bundles and the zones of amorphous material aggregations of thin microfibrils measuring 100–150 Å in diameter were observed When viewed in cross section they were seen to be assembled parallel with the fibre axis (Fig 3*b* 4)

Within the extracellular space membrane-bound corpuscles were seen containing amorphous electron-dense material and perimembranous haloes They could be located either in otherwise empty niches or in between the collagen fibril bundles No relation was found to the microfibrillar elements Vesicles ap-



come easily dislodged post mortem (Wright & Hubbard 1978) and numerous single otoconia tend to fall off the membranes during the process of dissection These types of artifacts are usually easily identified, in contrast to those which may be caused by the various agents used during the processing of labyrinthine tissue Werner (1934) first reported that fixation at low temperatures (10°C) dissolves the saccular otoconia, while the utricular otoconia remain intact. His observation is still unconfirmed by other investigators It was initially felt that the now established technique of microdissection which involves staining of the inner ear with neutral osmium tetroxide solution left the otoconia undisturbed Later several investigators expressed concern about the possibility that the subsequent dehydration and storage in alcohol may alter the otoconia. Lim (1973*a*) actually published a photomicrograph of collapsed' otoconia which had been stored for one week in alcohol The question of the possible occurrence of artifacts due to the processing of temporal bones for microdissection remains unanswered. Our feeling is that as long as all solutions including the alcohol are kept neutral the process does not significantly alter the otoconia. It should be kept in mind however that alcohol becomes oxidized and acidic with time The small amount of buffer remaining from the fixation solution is usually sufficient to maintain a neutral pH for some time We believe however that long term storage in alcohol should be avoided if a study of the otoconia is intended. At present we prefer to examine the otoconial membranes at least in a part of our material within a day or two after fixation.

METHODS

The specimens were stained with Millonig or Zetterquist's osmium tetroxide solution (pH 7.2–7 4) Some specimens were stained for 1 to 24 hours while others were stained for to 4 hours or less After staining a partial dehydration was performed with ascendin

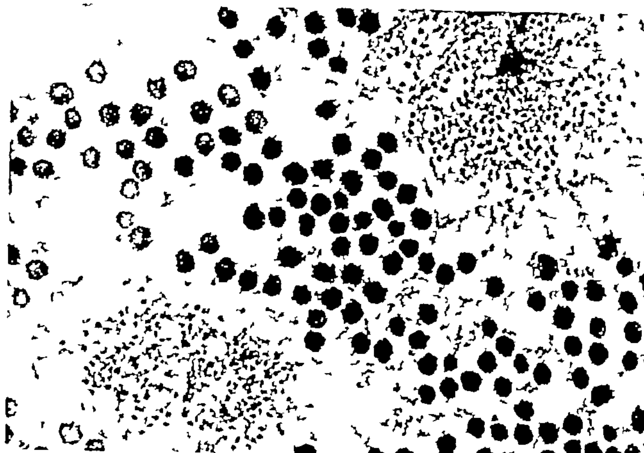


Fig 4 Cross-section of oxytalan microfibrils in between collagenous fibrils (×76 440)

tissue Regarding the collagenous fibres we found both disintegration increased fibre accumulation and in addition amorphous electron-dense material probably degraded collagen

The microfibrillar aggregations seem to be identical with the oxytalan fibres (for ref see Fullmer et al 1974) Evidence for the identification of these fibres is based on their histochemical properties and on the ultrastructure of their microfibrils (thickness approx 100–150 Å)

Because of their histochemical and enzymatic characteristics oxytalan fibres are accepted as structural glycoproteins which are strongly cross linked (Fullmer et al 1974) The physiological significance of these fibres seems to be an intermediate element of fibrogenesis which can hardly ever be detected in normal specimen Therefore the focal accumulation of oxytalan fibres in the tympanosclerotic tissue must be assumed to be a s of a pathologic ground substance with distu ance in the cross-linkage of the microfibr According to Winkle et al (1973) the collag fibre increase is similar to the oxytalan fil production primarily the result of a disturb extracellular fibrogenesis and less the result an augmented cellular activity

The presence of cellular debris and matı vesicles is also characteristic of tympan sclerosis The lysosomal nature of the matr vesicles has been demonstrated in other ti sues (Riede & Staubesand 1977) As wi other tissues the matrix vesicles in tympan sclerosis may be sequestered from the cell in the ground substance (Rabinovitch & Ande son 1976) or result from cell degeneratio (Spycher et al 1969 Tyberg 1974) The may be classified as Type I or Type II matri vesicles the latter showing a strong affinity t calcification

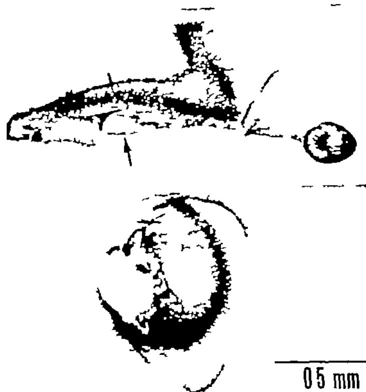


Fig. 5. The gelatinous layer of the otoconial membrane, right ear, from the macula sacculi (*top*) and macula utriculi (*bottom*). No normal-appearing otoconia are present, leaving the translucent membranes exposed with the reinforced, darker area above the striola clearly seen in both specimens. A single huge 'otolith' consisting of a conglomerate of calcite is present on each membrane. In the saccule the mass is attached to the accessory membrane (*top right*). Arrow indicates the original site of the otoconial mass. OsO

this reinforced line was actually raised from the neuro-epithelium so that a shallow groove was formed on the underside of the membrane along the striola. Cross sections were not made of the membranes, therefore the precise relationship between the groove on the superior and inferior surface was not determined.

In guinea pigs the dorsal extension of the macula sacculi tended to be less pronounced than in man. The gelatinous layer of the membrane had a loose, flat appendix of irregular shape extending from the inferior margin just opposite the dorsal extension or from the corner where the anterior and inferior margins meet (Fig. 5). The length of this structure was highly variable. We are not sure whether it is present in all animals. Usually the appendix was shorter than the distance from the tip of the dorsal extension to the inferior margin, but in some animals it was longer and could extend posteriorly halfway along the macula. When the extension was long it rested along the inferior margin of the macula against the membranous wall to which it was sometimes slightly adherent. Lim et al. (1974) and Lim & Erway (1974) have referred to this structure as the accessory membrane. It is of interest to note that the accessory membrane was usually covered by a thin layer of otoconia, although it was not in contact with the neuro-epithelium.

Abnormal otoconial membranes

Differentiating between normal and defective specimens was simple and unequivocal. There were no borderline cases. In the animals with an incomplete set of otoconia, the defects were always obvious and more or less symmetrical. None of our animals had unilateral otoconial defects. Furthermore, the abnormal-

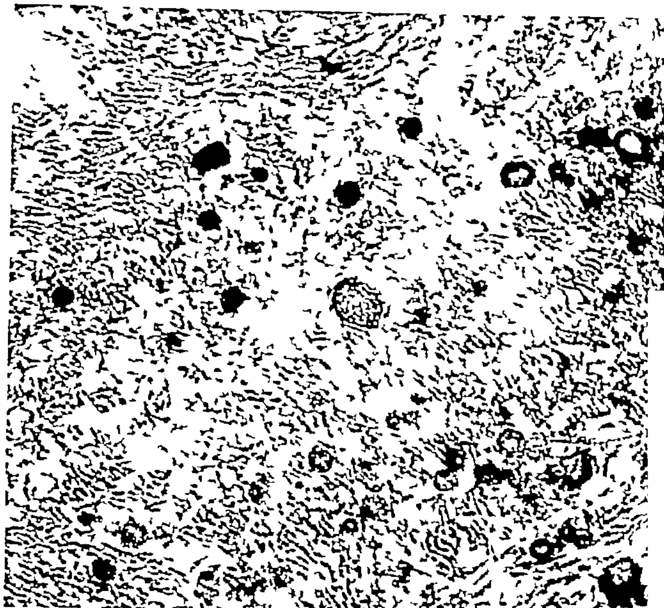


Fig 5b Multiple calcified matrix vesicles Type II (×5900)

in tympanosclerosis there seems to be such an alteration of the fibroblasts that lysosomes are released in increasing numbers into the extra cellular space *Such lysosomes are beyond* cytoplasmatic control and lead to a focal lysosomal activity In consequence there is a sur rounding cellular necrosis and a release of matrix lysosomes leading to a self perpetua tion of the inflammatory process Similar be haviour is described for the myocytic mediolysis and the chondrotic chondrolysis (Riede & Staubesand 1977) Underlining this hypothe sis for the causal pathogenesis of tympanosclerosis is the coincidence of D_3 hypervitaminosis and tympanosclerosis (Chang et al 1977) Under these experimental conditio[ns] the increased lysosomal activity is the basis [of] *the calciphylaxis (Riede & Staubesand 1977)*

As a working hypothesis the effect of suc[h] lysosomes beyond cellular control is twofold

1 There are changes in the fibrillar com ponents increased collagen formation deg radation of collagen and augmentation of oxy talan fibres These alterations are predomi nantly attributed to the presence of Type [I] vesicles Type I vesicles are lysosomes containing acid phosphatases Their enzyme ac tivity leads to degradation of the protein com-



r 6. Surface preparation of the membranous wall of t utricle shov the anterior part of the macula utriculi owing numerous otoconia attached to the wall and ntered on the surfaces of single cell Note to the right the sharp border between an area covered with otoconia and region free of crystals. Specimen from streptomycin-treated animal similar observations were made in untreated animals. OsO Phase-contrast.

)ne of these ears had been stored in 70% lcohol for 3 months The other specimen was issected the day after sacrifice It is highly nlikely that the finding represents an artifact. Each giant otoconium was formed by a conglomerate of calcite The utricular mass had a oundish main body with large crystals proruding from it. The saccular mass was also oundish with an uneven surface formed by nnumerable small sharp-pointed crystals

^{45}Ca incorporation in the defective otoconial membranes was variable but consistently lower than in normal-appearing membranes. It is not known whether the decreased uptake was due to the smaller total mass of otoconia in these animals or to a decreased calcium metabolism or other factors The defective specimens were not included in the ^{45}Ca study (Mechigian et al 1979)

No systematic examination of the macular neuro-epithelium was done Surface preparations of the neuro-epithelia were obtained from several abnormal-appearing maculae and examined in phase-contrast microscopy There was no obvious hair cell loss. No abnormal behavior or posture was observed in these animals However tests of vestibular reflexes or swimming ability were not performed.

Incidence of abnormality

In the hope that a type of animal could be found in which there was no occurrence of defective otoconial membranes a series of 3_ colored guinea pigs was examined. An attempt was made to correlate incidence of otoconial abnormality ... or (black vs dark

Thyberg J 1974 Electron microscopic studies on the initial phases of calcification in guinea pig epiphyseal cartilage *J Ultrastruct Res* 46 206
Weissmann G 1969 The effect of steroids and drugs on lysosomes In *Lysosomes in Biology and Pathology* (ed J T Dingle & H B Fell) vol I 276 North-Holland Amsterdam London
van Winkle W Hastings, J Ch Barker E. & Wohland W 1973 Role of the fibroblast in controlling rate and extent of repair in wounds of various tissues. In *Biology of Fibroblast* (ed E. Kulonen and J Pikkarainen), p 559 Academic Press, London, New York.

P D Dr W Mann
Univ -HNO-Klinik
Killianstr 5
D-7800 Freiburg
BRD

[l]ithic membrane is not known. Perhaps it may [h]elp to stabilize the otolithic membrane proper [o]r limit its movements. The otolithic membranes of the macula sacculi and utriculi in [m]an do not have accessory membranes (Johnsson & Hawkins 1967) nor does that of the [m]acula utriculi in the guinea pig. In gerbils and [p]igeons we have observed a prominent round [t]hickening of the anterior margin of the otoconial membrane in the utricle. This part of [t]he membrane is not covered by otoconia and [i]s in contact with the membranous wall.

In animals with both normal and abnormal otoconial membranes we found numerous crystals attached to the membranous wall of the utricle. They were particularly numerous in the abnormal utricle and were often seen clustered within the boundaries of single cells. The nature of these cells was not determined; probably they represented dark cells (Lim 1973*a*). These otoconia were examined only by light microscopy; admittedly it is difficult to evaluate their state so well as is possible in scanning electron microscopy. Because they were highly refractile, mostly small, and had the well-defined shape of normal otoconia, we do not believe that these crystals were in a state of decay like the ones pictured by Lim (1973*a*). On the contrary, it could be argued that the small otoconia were newly formed, but their origin and fate is enigmatic.

We did not observe any obvious tendency for the otoconia in the defective utricles to fall into the posterior ampulla and create a condition analagous to cupulolithiasis (Schuknecht & Ruby 1973, Schuknecht 1974). This would in any case be difficult to prove, since otoconia do become dislodged during processing and dissection.

It is hoped that this study will elicit further investigations of the otoconia in normal guinea pig populations. Perhaps the loss of saccular otoconia attributed to fixation in Werner's (1934) study did not represent an artifact—but rather pathology. Lindeman (1969) reported in his monograph on normal vestibular morphology that in some instances otoconial membranes had an unusual appearance and his brief description of these particular specimens suggests that they were similar to the abnormal specimens in our study. Harada & Sugimoto (1977) also noted that the otoconial layer was thinner above the striola region in the utricle of their untreated guinea pigs. They reported that a loss of otoconia occurred along this line after streptomycin administration. Their illustrations of this phenomenon appear similar to those seen in Figs. 2–4 in our untreated normal guinea pigs.

The high incidence of otoconial defects in the guinea pig makes this animal poorly suited for investigating experimentally induced otoconial pathology, and previous reports of otoconial abnormalities in guinea pigs should be viewed in the light of the present findings. On the other hand, a careful comparative study of the macular organs in the animals with normal and abnormal otoconial membranes could reveal important information about the formation of otoconia and the structures involved in this process. While the reduction in number of otoconia tended to be more random in the saccule, it always occurred above the striola in the utricle. The findings might imply a local disturbance of the calcium supply in this region due to some defect in the Type I sensory cells or in their supporting cells.

Assuming that sensory cells under the abnormal otoconial membranes can respond normally to stimulation, the two types of guinea pig could serve as a useful model in neurophysiological experiments concerning the effect of the mass of otoconia on the function of the macular organs.

ACKNOWLEDGEMENTS

This investigation was supported by USPHS Grants NS 11672 and NS 12706 and Program Project Grant NS 05785 and by a grant from the Leland Fikes Foundation to the University of Texas at Dallas subcontracted through the Aberrant Behavior Center, Dallas, Texas.



Fig 1 A phase contrast photograph of human spiral ganglion cells showing small neurons (arrows) and large neurons near the nerve fibers close to the osseous spiral lamina. The small neurons were followed in sequence from the beginning to end in serial sections.

before direct perfusion of 1% phosphate buffered osmium into the inner ear varied from 1-1/2 to 6 hours The procedure of preparing specimens for electron microscopy was described earlier (Kimura et al 1964) The bones were not decalcified and a drill was used only to thin the bony capsule of the apex before opening it with a pointed needle The specimens were embedded in Epon bisected along the longitudinal axis of the modiolus and each turn was separated out and divided into smaller pieces with a razor blade The modiolar part containing the spiral ganglion was separated from the organ of Corti at the osseous spiral lamina and the bone surrounding the neuronal masses was eliminated The specimens were initially examined under the phase contrast microscope then cut with an LKB ultrotome and photographed at 1000 to 30000 × magnification with a Siemens Elmiskop I A total of 17 bones from 12 individuals with an age distribution of 9 months 6 7 17 31 (two specimens) 37 42 59 65 75 and 92 years were examined None of these cases with the exception of the 59-year-old who had Meniere s disease had a history of known otological complaints Two specimens age 9 months and 37 years, were serially or semiserially sectioned and series of large composite electron micrographs were made Each neuron was followed in sequence to a thickness of more than 50 μm which was sufficient to include the width of the neurons

FINDINGS

A total of 971 human spiral ganglion cells was studied In direct contrast to the findings in small animals a majority of the neurons were unmyelinated There were essentially two groups of neurons large and small (Fig 1) Both myelinated and unmyelinated types were observed within each group The cytological details of the large and small neurons differed though a limited number of the small cells showed a cell organelle arrangement similar to the large ones

In our study the large neurons formed 94% of the population The perikarya were 22 to 34 μm in diameter and 22 to 64 μm in length They were mostly encapsulated by a single sheath of satellite cells (Fig 2A)

Acta Otolaryngol 89: 105–108 1980

THE MIDDLE EAR GAS COMPOSITION IN AIR VENTILATED DOGS

E. Ostfeld, J Blonder M Crispin and A Szeinberg

From the Departments of Otolaryngology and Chemical Pathology Chaim Sheba Medical Center Tel Aviv University Sackler School of Medicine Tel Hashomer Israel

(Received February 5 1979)

Abstract The middle ear gas composition has been examined in 5 air ventilated dogs under sodium thiopentone anesthesia. The gas samples are obtained by transtympanic puncture and analysed by gas chromatography The following mean ± S D gas composition was obtained: N_2 83 2±5 0 O_2 1..1±2.. and CO_2 4 7±0.7

The Middle Ear Gas (MEG) is an essential component for normal Middle Ear (ME) function Its quantitative alteration may cause pathological conditions such as the atelectatic ear (Sadé & Berco 1976) or the overinflation syndrome (Tjernström 1977) Both situations are related to a decrease or increase in the total MEG pressure Alterations in the relative composition of the MEG have focused little attention though recently Ingelstadt et al (1975) and Sadé & Weisman (1977*a* 1977*b*) showed that changes in the concentration of the individual gases oxygen (O_2) or carbon dioxide (CO_2) may be etiologic for some pathologic conditions. Quantitative and qualitative change in MEG occur under general anesthesia with nitrous oxide (N O) the accumulation of which has been described as being responsible for lateralization of the graft during tympanoplastic surgery neotympanum perforations and damage to the reconstructed ossicular chain (Thomsen et al 1965 Patterson & Bartlett, 1976) Those observations suggested the need for knowledge of the MEG composition under normal conditions and study of the dynamics of gas exchange in the ME by diffusion across the ME mucosa.

Very few and incomplete data are available on the composition of MEG (Drettner 1975 Elner 1976 1977) In this communication we present the results of the examination of the MEG composition in air ventilated dogs performed by a micro-method recently described by us (Ostfeld et al 1979)

MATERIAL AND METHODS

The MEG examinations were carried out on mongrel dogs Sodium thiopentone i v was used for anesthesia. During the procedure the dogs were ventilated with air by a Harvard pump through an endotracheal tube The respiratory rate was kept at 16–18 respirations per minute

The tympanic membrane (TM) was visualized through a window in the posteromedial part of the vertical portion of the cartilaginous external auditory conduct. This was performed by a retroauricular approach

Accurate visualization was achieved by magnification of an operating microscope through an ear speculum introduced into the shortened ear canal Only one ear was exposed during each experiment so that the head position was not changed and only healthy ears with non-perforated TM were included. Hamilton gastight syringes were used for transtympanic MEG sampling. Leakage of MEG or inadvertent admixture with the environmental air was prevented by performing the sampling under a seal of 4–5 mm saline covering the TM

Established Investigator of Chief Scientist's Bureau, Ministry of Health, Jerusalem.

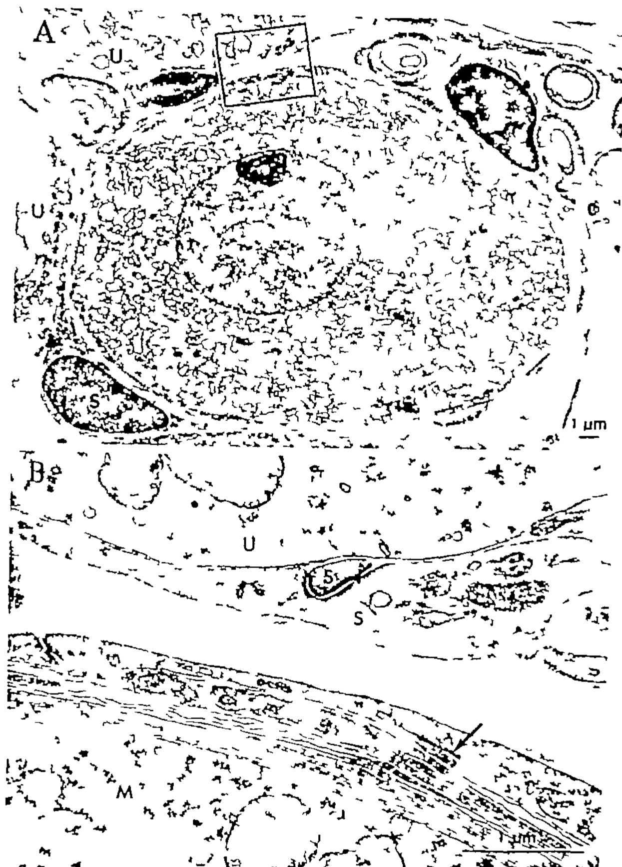


Fig 3 (A) A large myelinated neuron showing cytoplasm filled with mitochondria, free ribosomes and rough endoplasmic reticulum. Mitochondria are edematous due to post mortem changes. Myelinated neurons are often difficult to determine under low magnification Unmyelinated large neurons (*U*) Satellite cell (*S*)

(B) A higher magnification of the bracketed area in Fig. 3 A showing myelinated (*M*) and unmyelinated (*U*) neurons. Note a stack of desmosomes (arrow) in the myelin sheath and desmosomes between two satellite cells (*S*)

Acta Otolaryngol 89: 109–117, 1980

AUTONOMIC DYSFUNCTION ON THE AFFECTED SIDE IN MENIERE'S DISEASE

Takuya Uemura, Mitsuko Itoh and Naoko Kikuchi

From the Department of Otolaryngology, Tokyo Women's Medical College, Tokyo, Japan

(Received March 19, 1979)

Abstract. For ascertaining the existence of autonomic dysfunction on the affected side as being the cause of Meniere's disease, the mecholyl tests were conducted by means of conjunctival instillation in normal subjects as well as in patients with Meniere's disease, labyrinthitis and vestibular neuritis. The rate at which significant miosis (more than 10%) appeared in the mecholyl test was 3.1% among normal subjects, whereas the rate among Meniere's disease patients on the affected side was significantly higher during the attack, quasi-attack and interval stages. The appearance rate among Meniere's disease patients on the unaffected side was not different from that in normal subjects during any of the three stages. None of the patients with labyrinthitis or vestibular neuritis showed significant miosis on the affected side alone in the mecholyl test. This indicates that abnormality of vestibular-autonomic reflex in itself does not cause a positive reaction in the mecholyl test. Hence it can be concluded that the cause of Meniere's disease is related to the existence of autonomic dysfunction on the affected side.

Although it is generally believed that autonomic dysfunction is the cause of Meniere's disease (Williams, 1965), little evidence clearly indicating the presence of such abnormalities in patients suffering from this condition has been available. Uemura et al. (1972) examined the effects of mecholyl administered intramuscularly on the blood pressure of 20 patients with Meniere's disease. The positive rate, i.e. showing a response of either sympathetic hyperreactivity or sympathetic hyporeactivity (Gellhorn & Redgate, 1955; Redgate & Gellhorn, 1955), was found to be higher in these cases than in otitis media patients who complained of vertigo after ear surgery, as well as in normal subjects. The difference was particularly significant during the acute stage of Meniere's disease in which vertigo or nystagmus was present. However, on the basis of this study, we cannot explain the fact that most patients with Meniere's disease are affected unilaterally.

The pupils are innervated by the autonomic nervous system alone and they can react independently of each other. For these reasons we conducted mecholyl tests by means of conjunctival instillation to study the pupillary responses of patients with Meniere's disease and compared the results with those obtained from normal subjects as well as from patients suffering from labyrinthitis or vestibular neuritis.

APPARATUS

The binocular infrared pupillograph we have devised was used (Fig. 1). As shown in the block diagram (Fig. 2), the light from the halogen lamp goes through an infrared filter and illuminates each of the patient's eyes separately. After passing through the objective lenses and a half-prism, the images of the pupils enter the camera and the image intensifier simultaneously. The examiner can thus observe the pupils as displayed on the image intensifier. This equipment has the following characteristics. (1) Employment of an image tube allows visualizing and photographing of the pupil in dark infrared illumination, (2) combined images of both eyes eliminating the space occupied by the bridge of the patient's nose can be obtained through the use of the prism, and (3) the photographs can be taken while the patient is in a supine position. A motor drive

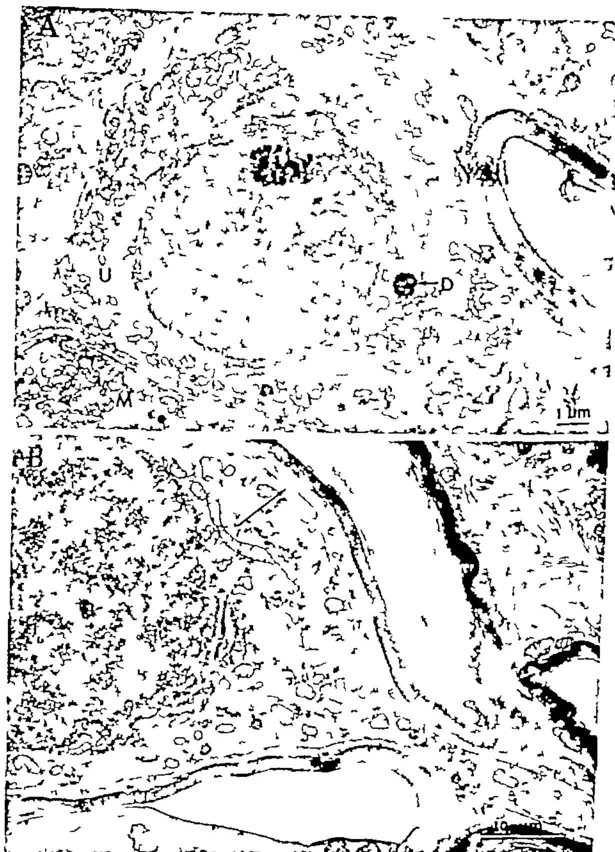


Fig 4 (A) A small unmyelinated neuron (*U*) adjacent to the small myelinated neuron (*M*) shows the filamentous cytoplasm. Mitochondria, free ribosomes and rough endoplasmic reticulum are relatively sparse The nucleus is round and the nucleolar substance s loosely organized Compare with the large neurons in Figs and 3 Dense inclusion (*D*). (B) A small unmyelinated neuron showing a dendritic process. Its satellite sheath contains an unmyelinated nerv fiber (arrow). The dendritic process shows a distribution of cell organelles similar to that of the perikaryon and the neurofilaments are not obvious

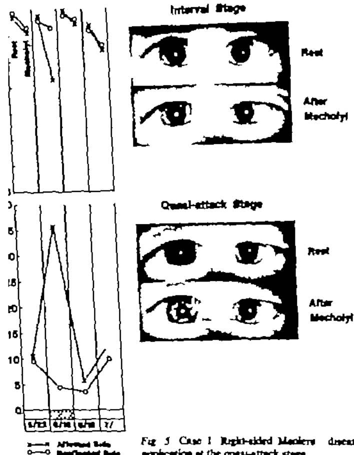


Fig 5 Case 1 Right-sided Meniere disease showing right miosis after mecholyl application at the quasi-attack stage

None of the 4 patients with vestibular neuritis who could be tested within 2 weeks after the onset showed pathological miosis of the affected side in the mecholyl tests, with one showing positive results on the normal side The severity of the vertigo in the 4 cases was equivalent to that at the quasi-attack stage in Meniere s disease In all 4 cases spontaneous nystagmus toward the normal side was present at the time of the testing and either no response or a severe canal paresis was revealed by the caloric test.

Case Reports

Case 1

H S a 50-year-old male with Meniere s disease on the right side was first seen on May 22, 1976 Two and a half years prior to the initial examination the patient had experienced attacks of rotary vertigo with tinnitus and hearing loss, both on the right side On June 16 1976 the tinnitus and hearing loss worsened, and spontaneous nystagmus toward the affected side appeared about an hour before the mecholyl test was started He felt as if an attack were imminent but did not have rotary vertigo (quasi-attack stage) The mecholyl test revealed obvious miosis on the right side However no difference between the pupillary diameters before and after mecholyl application was noted on the left side Three mecholyl tests were conducted on this patient before and after the quasi-attack stage but none showed any pathological miosis on the affected side (Fig. 5)

multipolar These neurons were fusiform or irregular in shape The size of the peripheral processes (dendrites) was larger or almost equal to that of the axonal process The myelin sheath when present was loosely organized and numbered up to 14 layers (Fig 5A B) The cytological characteristics in both myelinated and unmyelinated small cells were similar In the cytoplasm there were numerous neurofilaments in loose (Figs 4A 5B) and bundle forms which gave the impression of a paucity of cell organelles The density of filaments varied and the filaments were less obvious in a few cells Near the nucleus was an aggregate of vesicles and cisternae presumably Golgi network Dense cored vesicles were not evident One or two basal bodies were observed in a few neurons The nuclei were round and were located in the center or slightly off-center The nuclear chromatin substance was dispersed in a manner similar to that of the large neurons However the nucleolar substance of the small neurons showed an irregular shape and loose arrangement instead of the round or oval compact or dense forms of the large neurons Desmosomes puncta adhaerentia, were found between the sheath of the satellite cell and the plasmalemma of the perikaryon The sheath often contained one to three unmyelinated nerve fibers close to the perikaryon plasmalemma (Fig 4B) The small neurons were randomly located however there was a tendency for them to be located near the nerve fibers of the osseous spiral lamina or at the periphery of the neuronal mass (Fig 1)

DISCUSSION

From the present study as well as from our earlier ultrastructural studies of the human inner ear (Kimura et al 1964 1976) it has become increasingly clear that there are certain morphological differences in human ears in comparison to those of small animals The spiral ganglion is a prime example As stated earlier the majority of animal spiral ganglion cells are myelinated In our own study (Kimura, unpublished data) of rhesus and squirrel monkey inner ears the myelinated type was the most common It is difficult to conceive why such differences or almost a reverse proportion exists in the human since neuronal function is expected to be the same The possible significance may lie in conduction of neural impulses, that is the conduction rate in humans may be slower since the neurons lack myelin sheaths It is well known that the unmyelinated nerve fibers have a much slower conduction velocity than myelinated fibers (Tasaki 1953) Other factors which would influence conduction along the perikarya are the extent of unmyelinated portions of the dendrites and axons as well as the resistance provided by the neural surface and cytoplasm all of which are greater in the human than in small animals

No essential difference in morphology was noted in the satellite cell versus the Schwann cell and both of these cells are derived from the neural crest (Peters et al 1970) The presence or absence of myelin sheath does not seem to be related to neuronal size in the human since it was observed around both large and small neurons Maturation is evidently not a factor related to myelogenesis in the rat (Ross & Burkel 1973) In man considerable individual variation is noted in the distribution of myelinated neurons however there is a slight tendency for more myelinated neurons to be present in older individuals Unmyelinated neurons may become myelinated or simply the number of myelinated neurons increases due to a decrease in the number of unmyelinated neurons The population of human spiral ganglion cells is known to decrease in aged individuals (Schuknecht 1964 Suga & Lindsay 1976 Otte et al 1978) with or without a decrease in the sensory cell population The survival of neurons depends on environmental factors such as extracellular fluid

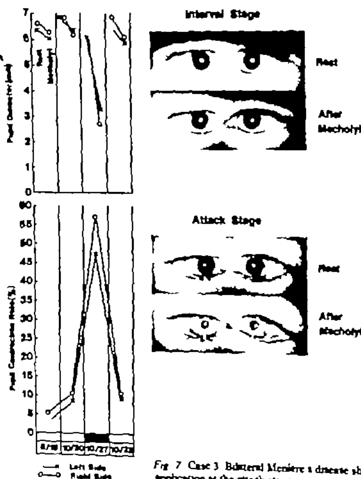


Fig 7 Case 3 Bilateral Meniere's disease showing miosis of both eyes after mecholyl application at the attack stage

Case 3

A M a 39-year-old female with bilateral Meniere's disease was first seen on July 31 1976 Three weeks prior to her first visit the patient had experienced a vertiginous attack with bilateral tinnitus and a sense of blocking of the ears. An attack of clockwise turning sensation accompanied by nausea began around 6 30 p m. on October 27 1976 Spontaneous nystagmus toward the right side was observed The mecholyl test conducted during the attack showed remarkable miosis in both eyes The three tests undertaken at the interval stage however did not indicate any abnormality (Fig. 7)

DISCUSSION

The mecholyl test by means of conjunctival instillation has been used to determine whether a large pupil is caused by sympathetic over activity or by parasympathetic paralysis. If the test shows significant miosis the case is diagnosed as the latter It is also said that miosis is more pronounced after a postganglionic than a preganglionic lesion This phenomenon may be explained by supersensitivity to acetylcholine or other related agents such as mecholyl developed at the area of the neuroeffector junction after section of a parasympathetic nerve The development of this supersensitivity does not occur immediately after

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Table I *Radiographical size of the mastoid air cell system*

R = right ear L = left ear B = bilateral ears

	Male				Female			
	No. of subjects	No. of ears	Mean ± S.E. (cm^2)	S.D.	No. of subjects	No. of ears	Mean ± S.E. (cm^2)	S.D.
Menière's disease	16	17	11.63 ± 1.29	5.31	11	11	8.20 ± 1.10	3.66
Other sensorineural hearing loss	16	16 (R)	13.31 ± 1.22	4.86	11	11 (R)	10.97 ± 1.27	4.21
		16 (L)	13.47 ± 1.08	4.31		11 (L)	10.35 ± 1.08	3.58
		32 (B)	13.64 ± 0.80	4.52		22 (B)	10.66 ± 0.82	3.83
Clinically healthy	13	13 (R)	13.29 ± 1.60	5.76	12	12 (R)	10.12 ± 1.05	3.63
		13 (L)	13.42 ± 1.54	5.56		12 (L)	10.12 ± 1.01	3.52
		26 (B)	13.36 ± 1.09	5.54		24 (B)	10.12 ± 0.72	3.50

ear healthy ($P<0.05$). No statistical differences were seen between male and female of Menière's disease, other sensorineural hearing loss and clinically healthy ears ($P<0.05$).

DISCUSSION

The correlation between poor pneumatization of the mastoid and chronic otitis media has been reported by several authors. Diamant (1940) suggested that the pneumatization of the temporal bone may be caused mainly by hereditary factors and "chronic otitis media" which tends to occur in such a situation. Tumarkin (1957) suggested that otitis media in intrauterine life or early childhood can disturb pneumatization of the temporal bone. Kollibova et al. (1972) planimetrically investigated the mastoid air cell system of the children who had had acute otitis media on at least one occasion and then compared them with those of normal children (Rubensohn, 1965). They found that the adequate treatment of the infection decreased retardation of the pneumatization of the temporal bones.

Meanwhile, Holmquist (1969) reported a correlation between the size of the mastoid pneumatization and the tubal function which is maintained to be closely associated with chronic otitis media. However, Andréasson (1976) insisted that the functional volume of the mastoid measured by physiological pressure technique is much more correlated with the tubal function because the mucosal damage of the middle ear and the mastoid can not be estimated correctly by measuring radiographical area.

The relationship between Menière's disease and the dysfunction of the auditory tube was reported by Tumarkin (1966) and Lall (1969). According to Tumarkin, the insertion of ventilating tube through the tympanic membrane is a rational treatment, on the basis of intermittent tubal obstruction of Menière's disease. However, Cinnamond (1975) disagreed with this theory.

In our study, we could not find any statistical differences of the mastoid pneumatization among Menière's disease, other sensorineural hearing loss and clinically healthy ears. This may at least mean that the infection of the middle ear in childhood is not the main aetiological factor for Menière's disease. Moreover, the size of the mastoid air cell system does not represent the size of the periaqueductal pneumatization, with reference to the report of Wilbrand et al. (1978).

The mean value of the radiographical area of the mastoid air cell system in Menière's disease was less than those in other sensorineural hearing loss and in the clinically healthy ears. However the differences were not statistically significant.

We cannot say whether the dysfunction of the auditory tube may be one of the aetiologi

the range of individual differences These figures are strikingly similar to those of the cat before section of the efferent nerve fibers (Spoendlin 1974) and our own study on the normal squirrel monkey (Kimura, 1978) Thus the same distribution pattern holds in the human as in animals however termina tion of dendritic processes of the neurons remains to be determined in the human

ZUSAMMENFASSUNG

Elektronenmikroskopische Untersuchungen des menschlichen Ganglion spirale wurden in 17 Ohren von 12 Individuen im Alter von 9 Monaten bis zu 9? Jahren durchgeführt. Zwei Arten von Neuronen und zwar große und kleine mit bestimmten cytologischen Merk malen wurden gefunden. Beide Arten waren sowohl myelinisiert als auch unmyelinisiert. Die Mehrzahl war unmyelinisiert (94%) Der Anteil myelinisierter Neurone variierte stark von Individuum zu Individuum doch eine Zunahme bei alten Personen wurde gefunden. Der größte Anteil war 28% bei einer Person im Alter von 75 Jahren Sechs Prozent etwa waren kleine Neurone Ihr Cytoplasma enthielt viele Neurofilamente sowohl in der myelinisierten als auch in der unmyelinisierten Art. Die vorliegende Untersuchung sagt nichts betreffend die funktionelle Bedeutung der Myelinisierung von Spiralganglionzellen aus.

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Robert S Kimura Ph.D
Departme t of Otolaryngology
Massachu etts Ey and Ea Infirmary
243 Charles Street
Boston Massachusetts 02114 USA

cifically selected for this study because they have been demonstrated to inhibit abducens motoneurons and because of their important role in nystagmus generation.

MATERIALS AND METHODS

Twelve cats weighing from 2.0 to 3.5 kg, were used Animals were anesthetized with halothane in oxygen through a cuffed endotracheal tube and mounted in a stereotaxic instrument. The animals were put in a supine position and using a ventral approach one labyrinth was surgically destroyed A bipolar stimulating electrode consisting of fine silver wires insulated except for their Ag-AgCl spherical tips was placed in the destroyed labyrinth The animals were then brought to a prone position with bodies comfortably supported on a plastic foam bed. The ipsilateral abducens nerve and medial rectus division of the oculomotor nerve were detached from their innervating muscles by an orbital approach and mounted on fine Ag-AgCl hook electrodes A small portion of the occipital bone was removed and the mid line cerebellum was aspirated to expose the floor of the fourth ventricle enabling a microelectrode to be inserted into the brain stem under visual control with the assistance of an operating microscope A pair of stainless steel screw electrodes were placed in the skull over the anterior sigmoid and posterior lateral cortices for recording EEG Bilateral pneumothorax was made to reduce movement of the brain stem. A catheter was inserted into the femoral artery and the arterial blood pressure and heart rate were continuously monitored on a large dial meter and intermittently on a strip chart recorder Rectal temperature was kept at about 37°C by a heating pad All wound edges and pressure points were infiltrated with 1% procaine The halothane was then discontinued and the animal was immobilized by intravenous administration of gallamine triethiodide (Flaxedil) under artificial respiration with room air Recording was not started until at least two h after discontinuation of halothane anesthesia.

Local anesthesia was maintained by repeated administration of procaine throughout the experiment. The animal was awake since alertness is necessary for the production of regular nystagmus which was the control condition in this study The effectiveness of the local anesthetic in controlling pain was carefully checked by observing changes of the heart rate (Lenox 1970) blood pressure (Kryzhanovsky 1976 Lenox 1970 Wolf & Hardy 1941) pupillary size (Kryzhanovsky 1976, Maeda et al 1972 Römer 1968) and EEG pattern (Maeda et al 1972) at rest. The animals were considered to be comfortable when the heart rate was regular in the range from 140 to 180/min blood pressure stable in the range of from 100 to 160 mmHg, pupil either in slit-like or mid-dilation and EEG showed 5–7/sec activity with infrequent 3 to 4/sec spindles. Furthermore appreciable changes on these parameters were not induced by tactile stimulation of wound edges On a few occasions when the local anesthetic was not renewed in time heart rate rose pulse pressure widened and blood pressure rose or became fluctuating and the pupil became larger Within seconds after injection of procaine into wound edges these symptoms returned to normal

After recovery from halothane anesthesia, most animals showed alternating rhythmic activity of the abducens and medial rectus nerves. These are the neural counterparts of regular vestibular nystagmus with the quick phase directed toward the side of the intact labyrinth In those instances where these patterns of nerve discharges were not regular or were replaced by tonic discharges 5 mg/kg of 1 amphetamine sulphate was given intravenously (Bradley 1968) In order to reverse the direction of nystagmus high frequency (usually 200/sec) electrical stimulation using 0 1 msec pulses was applied to the vestibular nerve on the labyrinthectomized side

Glass micropipettes filled with 2M NaCl so-

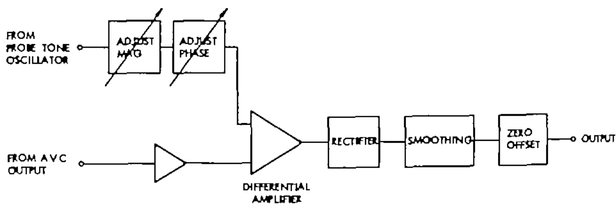


Fig 1 Additional circuit for admittance modulus measurement.

It is not sufficient merely to obtain the amplitude of this signal when dealing with measurements of eardrum admittance It is usually also necessary to compensate for the ear canal volume between the probe and the eardrum so that measurements are in dependent of probe placement When conductance and susceptance are available separately the conductance and susceptance of the ear canal initially obtained by tympanometry can simply be subtracted arithmetically from the conductance and susceptance measure during reflex contraction However it is no valid to do this in a *scalar* manner for th admittance modulus The errors involved i doing so for a probe tone frequency of 22 Hz are not normally large and many instru ment manufacturers accept this error How ever for the probe tone frequency of 660 H which is additionally available on th

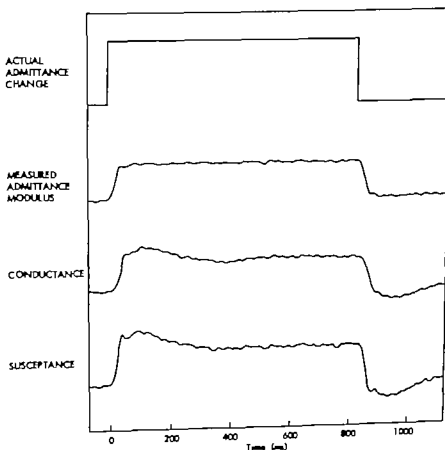


Fig 2 Transient characteristics of admittance modulus conductance and susceptance measures. The to trace indicates step changes in admittance whilst the three lower traces show responses of the instrumentation to this change. Note that only the conductance and susceptance traces exhibit overshoot.

n exactly opposite fashion Similar results an be seen in Fig. 6.

he early stages of anesthesia

The burst activity of BINs was clearly changed even in the early stage of nitrous oxide anesthesia. In all instances the changes were closely related to changes in the discharge patterns of the contralateral abducens and medial rectus nerves and in the EEG The most common change was a decrease in the maximum firing rate of each burst (28 of 30 neurons) In addition 21 of 28 BINs exhibited an increase in the burst duration and a decrease in the number of bursts per sec

The response of a typical neuron is shown in Fig. 3 The firing rate of this neuron (row 2 in inset figures) was recorded simultaneously with EEG (row 1) contralateral abducens nerve discharge (row 3) and blood pressure (row 4) The time course of changes in the burst activity was shown by successive measurements in 1 sec intervals of the maximum firing rate the burst duration and the number of bursts. In the unanesthetized control state characterized by low voltage fast waves in the EEG this neuron showed a high maximum firing rate of 600 spikes/sec This rate started to decrease gradually about 10 sec after the beginning of anesthesia. This *tendency to decrease* firing rate (A) lasted during anesthesia and was accompanied by a slowing and amplitude increase of the EEG The duration of each burst increased approximately linearly from 120 to 240 msec (B) In contrast, the number of bursts gradually decreased from 5/sec to 1/sec (C) BINs varied in their *resistance* to the effects of anesthesia. some were completely silenced after 20 sec others continued to show some bursting activity 240 sec after anesthesia onset.

Close inspection of abducens nerve discharge traces (Fig 3 row 3 in inset figures) shows that in the control record there is an abrupt cessation followed by a short term complete suppression of the nerve discharge at the quick phase This suppression was gradually replaced during anesthesia by a relatively long-lasting, weak suppression while the slow phase discharges were gradually prolonged and reduced in number and amplitude The envelope of the abducens discharge changed from a distinctly triangular shape in the control record to a much more rounded shape in anesthesia, corresponding to a decline in quick phase velocity

It should be noted here that, apart from the above changes no marked or consistent change in blood pressure was observed A level around the control value was maintained (row 4)

The changes *in a given BIN in its firing rate* burst duration and number of bursts described above were not invariably related to each other Three neurons exhibited a decrease in firing rate and an increase in burst duration without any clear change *in the number of* bursts. *In three other neurons* only a decrease in the BIN firing rate was observed In the remaining two neurons both *from the same* animal, the number of bursts *per sec* decreased during anesthesia, but the maximum firing rate increased. The burst *duration* remained fairly *constant In one cell* there was an increase in the number of bursts whereas the maximum firing rate decreased substantially In a few neurons the maximum firing rate increased and was accompanied by the usual increase in the burst duration and a decrease in the number of bursts/second

Yet even in the unusually behaving BINs described in the paragraph above there was still a precise relationship between certain discharge characteristics of these single neurons and the abducens nerve discharge Thus the increased firing rate of BINs was accompanied by an increased sharpening of the end of abducens slow phase discharge. The decrease in the number of bursts/sec was accompanied by a decrease in the number of nystagmus beats

The time to the first detectable change in either the maximum firing rate duration or number of bursts from the onset of anesthesia was measured for each BIN neuron and range

PROBLEMS AND PITFALLS IN OTOTOXICITY TESTING

Matti Anniko

From the Department of Otolaryngology Karolinska sjukhuset and King Gustaf V Research Institute Karolinska Institutet Stockholm Sweden

(Received March 6, 1979)

Abstract A local anaesthetic drug under development, intended for use in ear surgery was tested with regard to adverse, ototoxic effects Inner ear damage was not obvious. However in the guinea pig a massive formation of new bone occurred in the middle ear (bulla tympanica) within 5 weeks after a single local administration of the drug.

Since the introduction of the concept of ototoxicity in the early 1950 s research has often been focused on the effects on the labyrinth following the administration of aminoglycoside antibiotics given either systemically (Wersäll & Hawkins 1962 Wersäll et al 1973 review Hawkins 1976) or by local instillation into the middle ear (Wersäll et al 1969) During the 1960 s and 1970 s both environmental poisons (Falk et al 1974 Anniko & Sarkady 1978) and potent diuretics (Brummett et al 1977 Anniko 1978) have been shown to exert ototoxic effects under certain conditions

In the treatment of middle ear disease drugs with anaesthetic or antimicrobial effects have often been used locally e g Xylocain® injected subcutaneously or washing with sol borici iodine solutions etc To simplify the use of local anaesthetics in polyclinical work drugs having a rapid skin penetration have been developed which thus give complete anaesthesia following topical application This property is of great value for instance in the treatment of patients needing transmyringeal drainage A prerequisite is however that such a drug is atoxic with regard to the middle ear and inner ear structures

The present study is an analysis of one such drug under development and of whether or not it can exert ototoxic effects in the guinea pig when instilled into the middle ear

MATERIAL AND METHODS

The present series consisted of 30 healthy young guinea pigs (200 g) with a preserved Preyer s reflex and a normal tympanic membrane as observed in the operating microscope The animals were free from clinical signs of vestibular dysfunction *They were divided into three groups* each animal in the same group receiving 0 15–0.20 ml of either solution A B or C respectively The study

Table I *Table illustrating the composition of the test solutions A B and C*

Solutions A and B are patented by Astra Pharmaceuticals AB Sweden

Cod A	
100 ml contains	
Isopropanol	50 g
Glycerine	13 g
Conc. acetic acid	0.1 g
Water	28 g
Cod B	
100 ml contains	
Ketocaine	10 g
Isopropanol	45 1 g
Glycerine	11 6 g
Conc acetic acid	0 1 g
Water	[illegible] g
Code C	
Phys saline	
NaCl	155 mmol
Distilled water	1 000 ml

contralateral abducens nerve discharge, and by a progressive slowing of EEG

The results indicate that BINs exert the werful inhibitory effect on contralateral ducens motoneurons Even small changes in e firing of only one BIN were clearly re cted in the discharge pattern of the entire ducens nerve This powerful control could se from each BIN branching profusely and hibiting many of the motoneurons (Hikosaka : Kawakami, 1977 Hikosaka, et al. 1978*b*) r the uniform discharge pattern of all BINs Hikosaka & Kawakami 1977) or both.

The activity of most BINs was clearly suppressed by nitrous oxide This result is in contrast to those in studies of other central neurons At the same concentration of anesthesia as used here the spontaneous firing rate of spinal dorsal horn neurons was only moderately reduced in a brief initial period (de Jong et al 1970) and no consistent change was observed in the resting firing rate of thalamic relay neurons (Mori et al 1977) The depressant effect of nitrous oxide on BINs was not due to a decrease in blood pressure which has been reported to reduce the spontaneous activity of a class of brain stem neurons (Baust & Niemczyk, 1963) since no clear or consistent change in blood pressure was induced by nitrous oxide in the *present study*

The mechanism of nitrous oxide anesthesia is still not known. Recently it has been suggested that it releases or potentiates endogenous opiates (Berkowitz et al 1976) although other evidence puts this suggestion in some doubt (Smith et al., 1978)

Early in anesthesia, most BINs showed a synchronous decrease in *maximal firing rate* increase in burst duration and decrease in the number of burst/sec However a few BINs did not show this typical pattern In one instance an increase in the number of bursts was found, whereas the maximum firing rate decreased substantially In a few neurons, the maximum firing rate increased and was accompanied by the usual increase of the burst duration and a decrease in the number of bursts/second This dissociation in changes of the discharge parameters of BINs suggests that those parameters might be controlled by different neuronal elements which are simultaneously affected in most instances by nitrous oxide but independent in exceptional cases.

In the later stages of anesthesia, the firing pattern of typical BINs was changed from burst to tonic firing which was finally suppressed completely At the same time abrupt changes in activities of the eye motor nerves which indicate presence of the quick phase disappeared, and the discharge of the agonist nerve became tonic and the antagonist nerve became silent. Similar changes were observed in an additional two animals in which no labyrinthectomy was performed and horizontal vestibular nystagmus was elicited by sinusoidal oscillation of the turntable During nitrous oxide anesthesia, firing of BINs gradually *disappeared* and the abrupt changes in discharges of the opposing eye motor nerves were replaced by reciprocal spindle-like discharge patterns in phase with turntable oscillation. These results indicate that the *neuronal* mechanism generating the *quick phase is more* susceptible to anesthesia *than* that of the slow phase *Further* these results indicate that the primary and secondary vestibular neurons and ocular motoneurons were relatively *unaffected* by the anesthesia *to the extent that they were still capable of faithfully* responding to the angular acceleration stimulation According to Janeke et al (1969) the quick phase of the *vestibular* nystagmus is selectively abolished *by fentanyl* a morphine-like substance They speculated that the drug suppressed the quick *phase centrally* leaving the basic three neuron vestibulo-ocular pathway intact and that the *possible site* of the action of the drug is the *reticular* formation. Our results at the neuronal *level* support this speculation.

In the depths of anesthesia and at a *time* when BINs were silent, the abducens nerve showed tonic activity while the *medial* rectus nerve was virtually silent (Fig. 6 A) As anesthesia deepened *still* further the abducens


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Acta Otolaryngol 89: 135–143 1980

AN UNCOMMON APUDOMA
A FUNCTIONAL CHEMODECTOMA OF THE LARYNX

Report of a Case and Review of the Literature

E. Justrabo R. Michiels C. Calmettes[1] F. Cabanne[1]
H. Bastein J. C. Honot and J. Guerrin[2]

From the Department of Pathology Faculty of Medicine [1]Centre Georges-François Leclerc Dijon and the [2]Department of Nuclear Medicine Saint-Antoine Hospital, Paris France

(Received February 20 1979)

stract The authors report case of laryngeal chemo-dectoma in 53-year-old man who died from general semination with lymphatic and subcutaneous metastases This functional neoplasm secreted calcitonin vealed by fluorescence and biochemical tests and rhaps adrenaline-like substances. Its cells looked lik e light chief cells of the human carotid body they showed secretory granules and lysosomal formations. irty similar cases had been previously reported The perior laryngeal glomus and more occasionally the ferior ones were the seat of the these tumours which curred with an equal frequency in both sexes A arseness was often the unique symptom noticed ring the several months or years preceding the first amination of this slow growing neoplasm Neverthe ss among the chemodectomas of the head and the ck region those arising in the larynx had the highest cidence of malignant evolution Five of the patients resented general dissemination, two had lymphatic etastases and one local recurrence The ultra-structural features and sometimes the rarely detected functional activity of these chemodectomas are those of apudomas whose cells might originate from the cephalic ortion of the neural crests

Chemodectomas or paragangliomas are uncommon tumours of the larynx. They occur in the superior laryngeal glomus located in the supraglottic larynx or in the inferior one situated between the cricoid cartilage and the first tracheal ring (Lawson & Zak, 1974) A hoarseness, a dysphagia and/or a cervical pain of several months to years duration preceded the discovery of these slow growth neoplasms Unfortunately some of the chemodectomas are considered as epidermoid carcinomas In this paper we describe such a case whose issue was fatal. Speaking about 30 similar cases previously reported we should like to study the anatomo-clinical ultra-structural and functional features of laryngeal chemodectomas.

CASE REPORT

Clinical history

In 1973 a 53-year-old man was seen by his doctor for subcutaneous thoracic nodes At this time neither investigation nor therapy were prescribed A few months later in February 1974 a hoarseness appeared. An epidermoid carcinoma was diagnosed after a laryngeal biopsy in July 1974 Then a laryngo-epiglottectomy and a bilateral cervical lymphadenectomy were performed The left pyriform fossa was filled up with a 2.5×2×2 cm vegetant neoplasm which was destroying the homolateral false cord. The light microscopic examination confirmed the diagnosis of low differentiated epidermoid carcinoma invading two cervical lymph nodes A post operative radiotherapy was delivered on the two supra clavicle areas (5000 and 6000 rads) and on the nasopharyngeal field (5000 rads) In September 1974 multiple painful subcutaneous nodes were scattered all over the thorax the abdomen and the hips Several nodes were removed to relieve the patient and with a view to making a light and electron microscopic study


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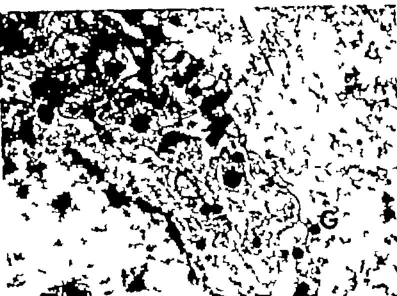


Fig 5 Typical secretory granules (*G*) were observed between the stromal collagen fibers and in the cytoplasm of tumour near by filaments (G ×8 600).

excretion gave the following results. adrenaline 135 µg/24 h and noradrenaline 390 µg/24 h (normal value adrenaline 4 à 8 µg/24 h and noradrenaline 25 à 50 µg/24 h). There was no variation of VMA (11 7 mg/24 h) Seventeen ceto and 17 OH steroids 5 H.I.A. calcemia and phosphoremia were unchanged.

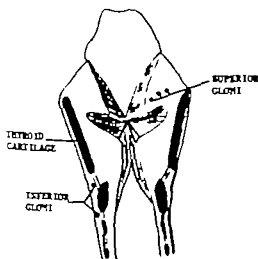


Fig 6 Topography of the laryngeal glomi.

CONCLUSION

The laryngeal tumour was a functional chemodectoma with lymphatic and subcutaneous metastases

DISCUSSION

Thirty cases of laryngeal chemodectomas have already been reported (Table I) This neoplasm has an equal sex incidence 15 cases have been discovered in men as well as in women with a maximum of frequency during the 5th decade of life. But two chemodectomas were individualized before the age of 20 in two young men, respectively 14 and 19 years old (Martinson, 1967 Ishida et al 1971) and in four patients over 60 (Hooper 1972, Tobin and Harris, 1972, Zachariah & Shah 1972 Hohbach & Mootz, 1978) Most of these tumours arose from the pair of superior laryngeal glomi whose diameter ranged from 1 to 3 mm According to Lawson & Zak (1974) these formations are located in the upper and anterior third of the ventricular fold adjacent to the superior margin of the thyroid cartilage Somewhat


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network and showed in one case only a central area of superficial ulceration (Adlington & Woodhouse 1972). On cut sections the fleshy soft tumour tissue was homogeneous or impaired by little necrotic foci.

It is interesting to point out that among the chemodectomas of the head and neck region those arising in the larynx have the highest incidence of malignant. While 18 patients had no recurrence after radical surgery, five others died with widespread dissemination in spite of surgery, radiotherapy and or chemotherapy (Adlington and Woodhouse 1972, Hooper 1972, Zachariah & Shah 1972, Personal observation 1979). A local recurrence was well treated by excision (Wockel et al. 1964) and two patients bore lymphatic and/or subcutaneous metastases at the time of the operation (Vetters & Toner 1970, Fraidooni 1976). The diagnosis of this uncommon neoplasm was often difficult. An epidermoid carcinoma was suspected three times (Hooper 1972, Fraidooni 1976, Personal observation 1979). A cylindroma, a parathyroid adenoma and hemangiomas were also identified (Andrews 1955, Gignoux et al. 1964, Azevedo-Gamas & Gloor 1968). But the notion of the slow growth, the radiation resistance of the tumour and a new histological analysis of the specimens should enable a rectification of the diagnosis.

In effect the structure of these chemodectomas was quite invariable with its nets, cords or trabeculae of cells and abundant hyalin stroma containing numerous vascular channels. All these tumours presented the same cellular features as revealed by electron microscopic study (Vetters & Toner 1970, Adlington & Woodhouse 1972, Piquet et al. 1976, Hohbach & Mootz, 1978, Personal observation). While the sustentacular cells and the nerve endings frequently found in the other chemodectomas were missing, the cells of these neoplasms were similar to the light chief cells of the human carotid body. They had a lucent cytoplasm with uniform spherical granules. The organelles which play an important part in the secretory process, the Golgi complexes, the microtubules, the filaments and the endoplasmic reticulum surrounded the granules and the lysosomal formations. The presence of microvilli and desmosomes is a further argument for the epithelial nature of the tumour cells which, with their granules, could secrete. But that functional activity of laryngeal chemodectomas was rarely proved. Only Vetters & Toner (1970) detected catecholamines in their study. The Falck test was positive and noradrenaline was extracted at a concentration of 0.145 µg/g. Our observation gives a typical example of functional chemodectoma producing calcitonin. Besides the unchanged VMA and the high values of the catecholamines obtained by the fluorimetric method are perhaps suggestive of "catecholamine like" secretion. All these data enable us to classify these chemodectomas in the apudoma group. These apudomas are tumours whose cells derive from neural crest elements belonging to the APUD *system* (APUD: Amine Content/or *Amine Precursor* Uptake and Decarboxylation) described *by* Pearse (1969, 1974). *The cells forming the* superior and *inferior* laryngeal glomi would come from the cephalic portion of the neural crests and would go along the X and the vagospinal nerves (Kissel et al. 1976, Perrin et al. 1978). So their tumours, as well as the other apudomas are rich in granules of neuro-secretory type and can secrete amine or amine precursor as in our observation.

RÉSUMÉ

Les auteurs rapportent un cas de chémodectome laryngé chez un homme de 53 ans décédé dans un tableau de dissémination générale avec métastases ganglionnaires lymphatiques et sous-cutanées. Ce néoplasme fonctionnel sécrétait un taux élevé de calcitonine ainsi qu'en témoignent les dosages biochimiques et l'étude en immunofluorescence. Il élaborait aussi, semble-t-il, des substances proches de l'adrénaline. Ses cellules qui ressemblaient aux cellules principales du glomus carotidien humain contenaient des grains de type neurosécrétoire et des formations lysosomiales. Trente cas sembl-

awaits further analysis The initial reaction post injectatem giving symptoms of vestibular dysfunction has no morphological grounds and is a reversible phenomenon Similar symptoms have been observed in clinical work when Xylocain® has accidentally been instilled into the middle ear (e g due to a perforation of the ear drum) Though these symptoms may last for a considerable time they are reversible leading to complete recovery

The bulla tympanica of the guinea pig is known to be very sensitive to irritant agents and it is possible that such a massive formation of new bone during so short a time—previously not reported in the literature—may be restricted to this species

When testing new drugs for the treatment of ear disease(s) it must be taken into consideration that they may primarily be relatively atoxic to inner ear hair cells yet exert considerable adverse effects on other adjacent structures thus impairing hearing *in toto* Secondary changes in the labyrinth cannot be excluded after a long interval

ACKNOWLEDGEMENT

This work was supported by grants from Karolinska Institutet and The Swedish Medical Research Council (grant no 12X 720)

ZUSAMMENFASSUNG

Ein neues lokalanästhetisches Präparat das jetzt für die Ohrenchirurgie entwickelt wird ist mit Rücksicht auf die ototoxische Effekte studiert. Keine Innenohrenschäden wurden gefunden. Aber im Mittelohr des Meerschweinchens entwickelte sich nach 5 Wochen eine massive Knochenstruktur nach einer einzelnen Verwendung des Präparates

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Matti Anniko M.D
Department of Otolaryngology
Karolinska sjukhuset
S 104 01 Stockholm 60
Sweden

Table 1 *Data on five patients having dyspraxia*

Patient	Age (years)	Symptoms and findings apart from dyspraxia	Diagnosis	Duration of speech disorders
1 (A S)	67	Asymptomatic hypertension + extrapyramidal components	Multifocal. Vasc. deg. of CNS	2 years
(S -E S.)	57	Inability to voluntarily activate cranial nerves	Multifocal degeneration process in CNS ALS?	2 years
3 (B K.)	37	Right sided hemiparesis	Left-sided anterolateral cerebral infarction	1 month
4 (R E)	68	Difficulties in memory	Presenile dementia	6 month
5 (N J)	4	Right sided hemiparesis	Subarachnoidal bleeding, aneurysm	months

Conventional electronystagmographic equipment (Henriksson et al. 1967 Henriksson et al. 1972) was used. The methods in brief were as follows Horizontal eye movements were recorded binocularly and vertical eye movements from each eye separately with a 4-channel DC-coupled pen-recorded (Mingograph 81 Siemens-Elema Stockholm Sweden) with an upper cut-off frequency of 15 Hz and an infinite time constant commercial electrodes (Medicotest®) with jelly were used The subjects were examined with the respect to

1 Command eye movements of 20°

2 Pursuit eye movements with a constant target velocity of 20° s and with a pendular target,

3 Optokinetic nystagmus in a wholefield optokinetic drum accelerating from a velocity of 0° to 60° s in 15 s

4 Vestibular nystagmus was studied with open eyes in darkness (*a*) induced by caloric stimulation with water of 30°C and 44°C for 20 s and (*b*) induced by angular accelerations of $120° s^{-2}$ for 1 s.

RESULTS

1 *Command eye movements*

The voluntary saccades were examined and compared with those of the normals (Fig 1) with respect to the following parameters

A *Accuracy* In four of the five patients there was a pronounced undershoot of the command eye movements which were each performed as two to four smaller saccades In one of the patients (No 4) there were also overshoots by the eyes passing the target by five to eight degrees

B *Fixation periods* The time intervals between the initiation of saccades of different directions were also studied In four of the five patients these periods were very irregular while in one case (no 2) these periods were significantly prolonged

C. *Velocity of saccades* The velocity of the saccades was also examined but did not show any differences from the normal subjects.

2 *Following eye movements*

Following eye movements were normal in patients and 5 and a slight disturbance was seen in patient 1 Gross abnormalities with irregular disruptions of the smooth eye movements by saccades were found in patients 3 and 4 (Fig. 2). Four of the normal subjects presented completely smooth eye movements while one of these subjects showed a slight irregularity in his tracking pattern

3 *Optokinetic eye movements*

The optokinetic nystagmus was within normal limits in all patients (Fig. 3) The fast and slow phases were consistently regular in both directions The amplitudes were however smaller and the frequency higher in

awaits further analysis The initial reaction post injectatem giving symptoms of vestibular dysfunction has no morphological grounds and is a reversible phenomenon Similar symptoms have been observed in clinical work when Xylocain® has accidentally been instilled into the middle ear (e g due to a perforation of the ear drum) Though these symptoms may last for a considerable time they are reversible leading to complete recovery

The bulla tympanica of the guinea pig is known to be very sensitive to irritant agents and it is possible that such a massive formation of new bone during so short a time—previously not reported in the literature—may be restricted to this species

When testing new drugs for the treatment of ear disease(s) it must be taken into consideration that they may primarily be relatively atoxic to inner ear hair cells yet exert considerable adverse effects on other adjacent structures thus impairing hearing *in toto* Secondary changes in the labyrinth cannot be excluded after a long interval

ACKNOWLEDGEMENT

This work was supported by grants from Karolinska Institutet and The Swedish Medical Research Council (grant no 1–X 720)

ZUSAMMENFASSUNG

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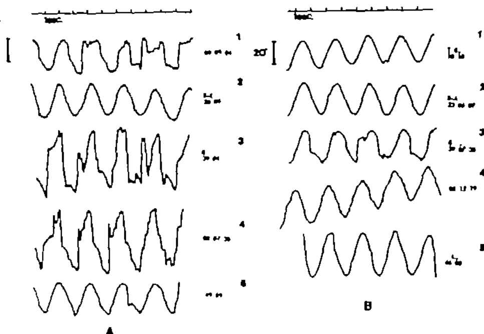
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Department of Otolaryngology
Karolinska sjukhuset
S 10401 Stockholm 60
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2 The following eye movements with pendular ... (A) patients with dyspraxia, (B) health con- ...

...rotatory and the caloric nystagmus were ...ak and of larger amplitude than in nor... ...ds.

Three of the patients showed disturbances ... pursuit eye movements These three ...tients had a pronounced increase in the ...plitude of the caloric nystagmus in rela- ...n to the other patients and also when ...mpared to normal subjects

DISCUSSION

A ...omparison between features of speech ... eye movements

...he pronounced relationship between speech ...isorder of the dyspractice type and disturb- ...nces in execution of command eye move- ...ents has not been previously described

In the highest centers speech would seem ... be processed in three different stages ... *a*) Development of purpose (*b*) integrated ...lanning; and (*c*) programming of the execu- ...ion of speech (Darley et al 1975)

Pure dyspraxia is defined as a disorder of the third stage About fifteen features have been described as typical for dyspraxia (Johns & La Pointe 1975) At least three such characteristics accuracy strength and tempo of speech all seem to have counter parts in voluntary eye movements

A *Accuracy* The dyspractic patients seem to be unable to accomplish the se quence of sounds which form the word. The word is frequently distorted by inaccurate sounds or syllables Because the sensory verbal systems in these patients are intact they will detect their error and try to correct the error with great effort

This inaccuracy in speech is reflected in the command eye movements by an inability to execute a saccade of appropriate size The saccade produced will be inaccurate frequently too small and (as in dyspractic speech) followed by corrections It will thus be split up frequently into two or more shorter saccades with or without overshoots

B *Strength* Strength of dyspractic speech may be normal with the words uttered with



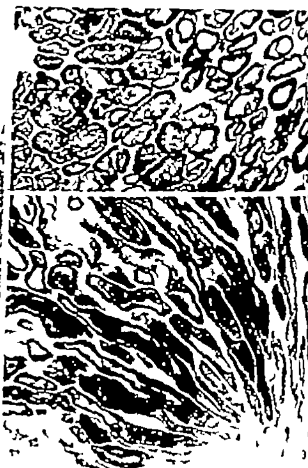


Fig 1 Biopsy of the cochlear nerve from a man with 8th nerve neurilemoma The crushed area in the central parts of the biopsy was brought about by pinching with a pair of forceps ×115

Fig Higher magnification of the site of crushing artifact shown in Fig 1 On the right the better preserved nerve fibres show myelin coats of normal thickness or occasionally slight thickening of the coat and splitting of the myelin layers On the left ample myelin material has replaced the axons which occasionally are seen as light areas among the myelin material ×702.

Fig 3 Longitudinal section of the site of compression The center of the injured area is at the right lower corner towards which the nerve fibres converge It appears as if the compressed myelin material had been forced along the lumen of the axis cylinders. No myelin is seen at the very site of pinching Superior vestibular nerve from a patient with Meniere s disease. ×541

almost totally displaced by thick myelin layer It often looked as if myelin material had slid away from the site of compression towards the periphery Axons were present in the area of compression but appeared fragmented or thinner than normal (Fig 3) Tangential sections showed occlusion of the nerve fibres with myelin material in the zone surrounding the center of compression Not only thick layers of myelin but also whorls and layers of myelin invaginating into the space between the axon and the myelin coat were seen a little farther from the very site of compression

Samples taken from the rat also showed greatly thickened myelin layers (Fig 4) and at the peripheral sites of damage also myelin whorls and invaginating myelin layers (Fig 5). The above changes were rarely seen in samples taken after fixation In places the axons were thicker than normal and in some areas the myelin coats had also ruptured

Electron microscopy showed denuded and fragmented axons among vesicular debris left by the myelin material in the areas in which the myelin material had been most violently damaged (Fig 6 A) In those areas Schwann cells were no longer seen or ruptured cells or free nuclei were detected In other places

...me daß willkürliche Saccaden in der Rinde des ...appens ausgelöst ...erden. Eine Vermehrung des ...abren Nystagmus in den beschriebenen Fällen ...im Zusammenhang mit einer Enthemmung auf ...d eines Wegfalles der cortikalen Inhibition auf die ...tät des Hirnstammes.

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N G Henriksson M.D
Department of Otolaryngology
University Hospital
S-221 85 Lund
Sweden

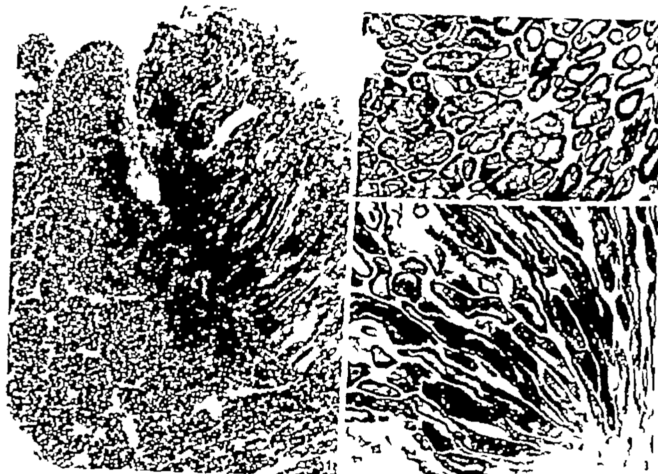


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Fig. 1. Cat nasal mucosa, maxilloturbinal area. Numerous beaded VIP immunoreactive nerve fibres around acini of seromucous glands (×300).

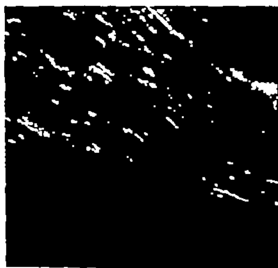


Fig. 2. Posterior nasal nerve. Oblique section. Coarse nerve fibres displaying VIP immunoreactivity of varying intensity are abundant in the nerve trunk (×200).

of both sides and processed for immunohistochemistry.

Immunohistochemistry

Specimens were frozen to the temperature of liquid nitrogen in a propane-propylene mixture and freeze-dried. They were then exposed to diethylpyrocarbonate vapour at 55°C for 3 h (Pearse et al. 1974) and embedded in paraffin in vacuo. Sections 6 μm in thickness were subjected to the indirect immunofluorescence method (Coons et al. 1955) or to the peroxidase-antiperoxidase (PAP) procedure (Sternberger 1974) for the demonstration of VIP. The VIP antiserum (code no 5603) was kindly supplied by Drs. J. Fahrenkrug & O. Schaffalitzky de Muckadell, Department of Clinical Chemistry, Bispebjerg Hospital, Copenhagen, Denmark. The antiserum has been characterized in detail elsewhere (Fahrenkrug & Schaffalitzky de Muckadell 1977). The antiserum was used in dilution 1:80 (immunofluorescence) or 1:5600 (PAP procedure) and the site of the antigen-antibody reaction was revealed by fluorescein isothiocyanate labelled sheep antirabbit IgG (SBL, Stockholm, Sweden) diluted 1:20 or by PAP staining. PAP complex (Cappel Labs, Downington, Pa., USA) was used in dilution 1:320. Immunofluorescence was observed in a fluorescence microscope with filters selected to give peak excitation at 490 nm. PAP stained sections were examined in a light microscope. Sections incubated with antiserum inactivated by the addition of excess antigen (100 μg of pure porcine VIP per ml diluted antiserum) served as controls.

RESULTS

VIP immunoreactive nerves, mostly in the form of delicate beaded terminals, were numerous in the nasal mucosa. The nerves were particularly frequent in the maxilloturbinal area. Scattered VIP fibres were also observed in the septum as well as in the ethmoturbinal and nasoturbinal area. Generally the VIP nerves were distributed beneath the surface

with apparent thickening of the myelin coat there was separation of the myelin lamellae (Fig 6B) The site of separation varied but usually occurred at the minor dense line (Fig. 6 D) There were areas of swelling around the axoplasm (Fig 6 B) Because these areas showed two membranes on the axonal side the swelling was probably in the adaxonal Schwann cell cytoplasm Such swelling was also seen in samples taken after fixation but it was much more common in biopsies taken before fixation The separated myelin lamellae were roughly parallel and this pattern was also common in those surgical biopsies which showed compression artifacts In the latter aggregates of artifactually separated convoluted myelin layers were seen also (Fig 6 C) The axon was found to be smaller than usual at the periphery of the fibre The latter changes however were problematic to interpret These biopsies also showed changes which most probably were of pathological nature Because the change was more common in biopsies which showed distinct light microscopic evidence of compression artifact than in biopsies which did not it is probable that the change at least partly was caused by compression

DISCUSSION

Artifactual changes corresponding to those we found after deliberate rough manipulation of tissue described above were also noted after the most careful handling of tissue where the nerve tissue was sliced with a razor blade before fixation with glutaraldehyde (Dyck 1975) In both these situations the myelin sheaths appear thicker than normal and show separation of myelin lamellae Corresponding changes are also shown in the micrographs by Schlote (1971) in his study on traumatic changes of the optic nerve The myelin sheath may also show abnormal ruptures and increased numbers of myelin whorls inside the sheath

In certain pathologic conditions such as trigeminal neuralgia and hemifacial spasm degenerative hypermyelinisation has been described (Beaver et al 1965 Kumagami 1974) Such changes are very difficult to distinguish from artifacts as shown by our study In the above conditions other changes also occur (microneuromas which were not seen after mechanical damage) suggesting that the above-described changes are real Cadmium chloride induced neuropathy (Sato et al 1978) may also present nerve fibres with thickened myelin coats If mechanical damage occurs before fixation these changes may be hidden behind artifacts On the other hand, numerous artifactual changes in the nerves may suggest degenerative hypermyelinization Under certain conditions interpretation of morphological findings may thus be extremely problematic Additional methods such as the analysis of teased preparations might then be helpful The only way of avoiding these artifacts is to avoid mechanical handling but we hope that this description of artifacts may be of some help in cases in which mechanical handling cannot be avoided

It should also be remembered that fixation may cause artifacts in addition to those caused by handling of unfixed tissues Beaver et al. (1965*a* *b*) demonstrated fixation artifacts in human trigeminal ganglia after glutaraldehyde fixation Artifacts due to fixation may also appear in perfusion fixation (Berthold 1968) and it appears that a careful selection of the fixative to be used is necessary in each study Various alternatives should be tried and the one giving the best preservation should be chosen

We compared our findings with those described recently by Balentine (1978) in experimental spinal trauma He found axonal fragmentation 30 min after trauma We also saw evidence of axonal fragmentation He observed adaxonal swelling in his biopsies as did we Likewise he noticed this change in the controls also This suggests unspecificity of adaxonal swelling The change is possibly caused by fixation and occurs more often in samples already damaged mechanically Ve-

ACTA OTO LARYNGOLOGICA
Vol. 89 No 3–4

COMPTE RENDU
DE LA RÉUNION SCIENTIFIQUE DU

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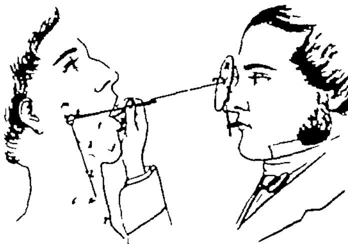


Fig 5 Investigation of larynx with mirror as proposed by Czermak

prize I feel it was necessary to mention that he came from a Hungarian family

In 1873 Heinrich Neumann was born in Héthárs also in Hungary He studied in Vienna and the Otologic Section of Francis Josef's Ambulatorium was at one time under his direction and later he was director of the University ENT Clinic in Vienna.

Finally I must say some words about the Nobel-prize winner Gy Békésy who died 7



Fig 6 A. Politzer



Fig 7 Gy Békésy

Acta Otolaryngol 89 76–84 1980

EVIDENCE FOR SOUND PERCEPTION WITH THE LABYRINTH

J D Bleeker H P Wit and J H Segenhout

From the Department of Otorhinolaryngology and the Institute of Audiology University Hospital Groningen The Netherlands

(Received February 5 1979)

Abstract A report is presented of experiments on guinea pigs and pigeons concerning behavioural and electrophysiological responses of the labyrinth to sound In the guinea pigs the cochlea was destroyed on both sides by CO_2 laser radiation and fenestration of the lateral semicircular canal was done In the pigeons the cochlea was removed before fenestration On account of the results obtained fenestration of the lateral canal in patients with bilateral total cochlear loss can be considered as an alternative to monopolar cochlear implants

Total cochlear loss on both sides is a serious handicap—even more so when vision is also severely impaired or totally lost In such cases one is deprived of remote information and is nearly totally dependent on communication through touch and deep sensibility

Helen Keller (1902) gave us in "The Story of My Life" a stirring account of her feelings and the loneliness to which these patients are doomed She described how very important every scrap of information is however meagre and how other senses in fact the touch can be trained to perfection in order to obtain information from outside This information however especially that obtained by touch is limited to direct contact

One of the methods used in an attempt to help these patients is direct stimulation of the cochlear nerve with cochlear implants This treatment has disadvantages we need not discuss here (Bilger 1977 Flottorp 1976 House 1967 Tonndorf 1977 Tonndorf et al 1979) Especially for the multiple electrode implants a rather extensive operation is needed (Chouard 1978)

One might wonder whether the phylogenetically older sense organ for perception of low frequency vibration in the surroundings, the labyrinth can obtain sonic information when adequately exposed to sound stimuli There are arguments to support this assumption

(*a*) The fenestration operation frequently performed 30 years ago in cases of stapes fixation due to otosclerosis renders the maculae in the vestibule and the crista in the lateral canal accessible to sound vibration in air—especially when unlike the original fenestration operation the tympanic cavity and the impedance matching of the middle ear can be left intact

(*b*) The Tullio reaction demonstrates that after fenestration a labyrinthine stimulus can be evoked by sound

(*c*) Earlier experiments on pigeons have shown that after the cochlea has been eliminated a microphonic effect can be obtained by fenestration of for example the lateral canal this effect can be abolished by damaging the ampulla and the maculae in the vestibule (Bleeker 1949 Bleeker & de Vries 1949 de Vries & Bleeker 1949 van Eyck 1955)

(*d*) Vestibular neurons of squirrel monkeys and fenestrated deaf mice show responses to audio-frequency sound (Young Fernandez & Goldberg 1977 Mikaelian 1964)

(*e*) In cats and monkeys a cortical projection of the vestibular system has been demonstrated (Ödkvist et al 1977 Schwarz & Frederickson 1974) So far as we know this has not been confirmed in man But certain neurological disorders such as astereognosis are

SYMPOSIUM

Immunological Problems in Oto-Rhino-Laryngology

Moderator: L. Surján

Opening Remarks

During the last few decades modern immunology has grown into a completely new branch of medicine. Present-day immunological research is concentrated on establishing the site and mechanism of antibody production, on the structure and biological activity of antibodies and on the cellular and molecular basis of immunity. The organism is exposed to the influence of its environment. Various kinds of infections, antigens and allergens invade the mucous membrane and skin. We meet 'foreign' materials of an antigenic or allergenic nature from the first breath and swallow to the last, that is, from birth to death. Therefore throughout our life the recognition of these 'self' and 'non-self' materials is of basic importance. The homeostasis is based on a well organized defence mechanism, which defends the organism whether or not those 'non-self' materials are of an infectious nature.

The organism has mechanical (hair cells), biochemical (enzymes) and immunological defence mechanisms.

The main surfaces on which the organism is in contact with antigens and allergens are the mucous membranes of the respiratory and digestive tracts, a great part of which lie within the field of oto-rhino-laryngology. Therefore we must have a wide horizon to see and judge not only the local reactions and symptoms but also the general ones.

The problem caused by allergic reactions will not be discussed today.

I believe that the basic tenets of immunology are well known to the Members of Collegium and it is therefore superfluous to repeat them on this occasion. On the other hand it would seem necessary to mention the following few observations or impressions connected with otolaryngology.

(1) I feel that generally, from an immunological point of view, oto-laryngology is on the decline compared with other clinical branches. However, one of the important lymphoid organs, the Waldayer-ring, comes within our field and, as was mentioned above, the mucous membrane of the respiratory tract represents a very extensive surface on which we meet different kinds of bacteria, viruses, etc.

Some of the immunological and inflammatory reactions are observable within the field of oto-rhino-laryngology.

(2) It is necessary to take into consideration that it is not easy to follow the very rapid development of immunology.

Oto-laryngologists are much more influenced by surgical techniques, by the good or apparently good effects of antibiotics, etc., than by immunological knowledge. In consequence, we are not up-to-date in immunology.

(3) A very serious and common mistake is also observable. Classical immunology deals with specific cellular and humoral reactions of the body under the influence of a specific antigen, e.g. tetanus, diphtheria, smallpox, etc., and also their theoretical problems, but not with the inflammatory reactions caused by bacteria or by the common viruses. The immunological and inflammatory reactions have some similarity but they have far more differences. The inflammatory reactions are also connected with the actions of immunoglobu-

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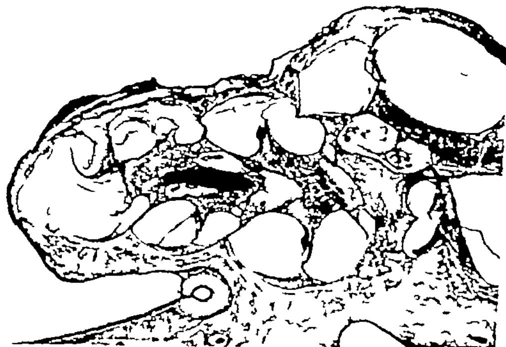


Fig 3 Cochlear destruction after laser radiation. On the surface laser spots The organ of Corti is destroyed

stimuli In this way threshold determinations are possible

In two stages with an interval of 2 weeks the left and the right cochleae were destroyed The animals were brought under general anaesthesia with a gas mixture of oxygen (1.5 l/min) and fluothane (1.5–2 %) a simple and in our experience more reliable method than the often-used anaesthesia with intraperitonial sodium pentothal (Hoar 1969) [1] On the ventral side slightly medial to the mandibular angle the bulla was exposed and opened An equal response curve of 10 μV of the cochlear microphonic was recorded with a single electrode of 50 μm nichrome wire on the outer part of the first turn near the round window Next the compound action potential for 3 000 Hz tone bursts of 3 msec duration at a rate of 10 per second was registered on an oscilloscope screen (Fig 1) Sound pressure was measured with a 1 inch condensor microphone placed close to the animal's ear The cochlea was then destroyed by CO_2 laser radiation Seven shots of 1/10 sec duration were given with an output power of 10–20 Watts depending on the local thickness of the wall of the cochlea We chose the laser for destruction of the cochlea in order to minimize the damage to the labyrinthine structures The laser radiation caused total destruction of the organ of Corti with extensive damage to the vascular striae in particular (Figs 2 3 3*a*) Two or three weeks after destruction of the second cochlea the animal was fenestrated Prior to the operation the nystagmogram and the shiver audiogram were checked The nystagmogram should be normal the Preyer reflex negative and the shiver audiogram without significant pauses at sound intensities of 100 dB (SPL)

At operation the bullae were opened retroauricularly and the middle ear mucosa was inspected for inflammatory reactions Next an electrode was placed on the bony wall of the lateral canal near the round window and we checked whether any microphonic or an AP could be registered at maximum sound intensity of about 100 dB (SPL) The noise level of our first set-up made detection below 5 μV

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[1] Laboratory data of the arterial blood after hours narcosis (compared with normal values) pH 7 159 (7 471), P_{CO_2} 5 69 kPa (3.81) P_{O_2} 56.85 kPa (10.56) sat 99 7 % We thank L. Prins and J Bartels for their help in obtaining these data.

THE FIRST LINE OF DEFENSE

(MODIFIED AFTER DATA FROM T.B. TOMASI AND P. BRANDTZAEG)

SURFACE OF THE MUCOUS MEMBRANE

I PHYSICAL CLEANSING BY THE MUCOCILIARY TRANSPORT SYSTEM

II UNSPECIFIC PROTECTIVE FACTORS

FUCOMUCINS,
SIALOMUCINS,
SULFOMUCINS
(GLYCOPROTEINS OF THE MUCUS BLANKET)

LYSOZYME
LACTOFERRIN
SECRETORY GLUCOSIDASES
INTERFERON
COMPLEMENT
SECRETORY PROTEASE INHIBITORS

III SPECIFIC FACTORS

IMMUNOGLOBULIN
IMMUNOGLOBULIN
IMMUNOGLOBULIN

PROTECTION AGAINST INVADING MICROORGANISMS
NEUTRALIZATION OF VIRUSES
NEUTRALIZATION OF TOXINS
OF BACTERIA,
OPSONIZATION

Fig. The first line of defense (modified after data from T. B. Tomasi and P. Brandtzaeg).

– as a *response to a virus infection in the infect*ed cells and then prevents further intracellular replication of these microorganisms. Since this agent is available in the affected region only a few hours after a virus infection, it is also capable of being distributed extracellularly on a relatively large scale in and with the secretion. This enables it to keep pace with the viruses which are also replicating and spreading very quickly and to inhibit if not prevent further replication.

The action of interferon is manifold (Fig. 4): although it cannot prevent viruses from pene*trating into somatic cells* it can prevent the intracellular replication and spread of very many virus types and even a few other microorganisms. In addition interferon is a lymphokine and activates the phagocytic activity of the macrophages in the cell-linked immune response. Moreover this protective substance is quickly available in case of infection whilst it is a well-known fact that specific antibody production does not begin until several days thereafter. The high and polyvalent antiviral effectivenes of interferon has been clearly demonstrated by experimentation in particular in infections of the upper respiratory tract. Unfortunately interferon is not yet available in sufficient quantities for large-scale clinical use or for more than testing in different virus infections. One of the main problems *at present is to find suitable* atoxic inductors for interferon synthesis in man.

Another protective factor present in the nasal mucosa and secretion is complement.

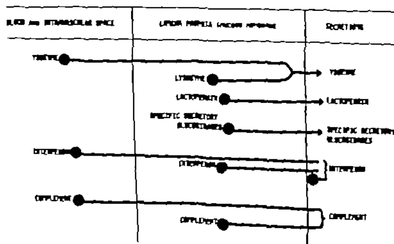


Fig. 3. Unspecifically acting proteins in nasal secretions.

R 1

	bef re fen t ion	f 1	f ne AP	ion hive	
2					
3					
4	†				
5					
6					
7				†	
8	†				
9					no pre-ope tive check f shive
10					
11	†				
12	†				
13	pos				
14	†				
15					←
16	res ffect				
17	†				
18					←
9	†				
20	n/h ffect				

Fig 5 Experimental results of 20 guinea pigs 7 animals died before fenestration 4 had to be eliminated The 2 animals with positive results are indicated by arrows

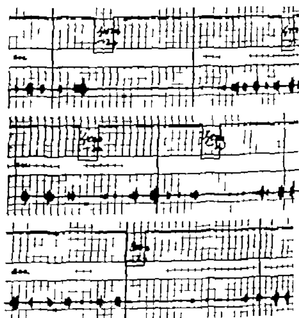


Fig 6 Shiver audiogram of guinea pig no 15 after fenestration Prior to fenestration there was no response Frequency and relative level of the tone burst are indicated in the sound registration line.

are accessible After opening the recessus scalae tympani the ductus cochlearis can be removed as a whole with a small hook Fenestration is performed by shaving a hole in the exterior part of the lateral canal with a small chisel Care must be taken not to damage the large blood vessel accompanying the canal

Before and after removal of the cochlea microphonic and compound action potentials were recorded with a single electrode (50 μm nichrome wire) placed through the hole in the recessus scalae tympani After fenestration recording of the electrophysiological signals was repeated with the electrodes still in the same position as before the fenestration

Schematic arrangements of the apparatus for the electrophysiological measurements are given in Fig 4 (this apparatus became available to us after the completion of the guinea pig measurements) Acoustic stimuli were generated with a Telephonics TDH 39 headphone and lead to the pigeon s ear through a plastic tube Sound pressure calibration was done with a calibrated miniature microphone by connecting this tube to a small cavity instead of the ear of the animal In most of the action potential measurements the acoustic stimuli were presented with alternating phase in order to cancel the microphonic component in the signal through averaging After proper amplification the signal from the electrodes was fed to a modified Princeton Applied Research type TDH 9 Signal Averager No filtering was applied to the signal during the action potential measurements

The memory content (100 points) of the analogue averager was written out with an XY recorder after smoothing the signal by means of a low pass filter At the end of the measurements some animals were sacrificed for microscopic examination The others are still alive and kept for cochlea exturpation on the other side Further experiments on behavioural responses will possibly be done with these pigeons (Harrison & Furumoto 1971).

RESULTS

Guinea pig experiments

The first program has so far been completed in 20 guinea pigs (Fig 5) The extensive pro-

IE ECOND LINE OF DEFENSE
(MODIFIED AFTER DATA FROM T B TOMASI AND P BRANDTZAEG)

LAMINA PROPRIA

UNSPECIFIC FACTORS

INTERCELLULAR SUBSTANCE AND FIBRILS
MICRO- AND MACROPHAGES
MASTCELLS
VESSELS
AUTONOMIC NERVOUS SYSTEM
HORMONES
(COMPLEMENT INTERFERON
PROTEASE INHIBITORS ETC)

II SPECIFIC FACTORS

SENSITIZED B- AND T LYMPHOCYTES
EOSINOPHILIC LEUCOCYTES
IMMUNOGLOBULIN G
IMMUNOGLOBULIN M
IMMUNOGLOBULIN E
(IMMUNOGLOBULIN)

NEUTRALIZATION OF VIRUSES
LYSIS OF BACTERIA,
NEUTRALIZATION OF TOXINS
OPSONIZATION,
FORMATION OF IMMUNO-
COMPLEXES,

Fig 10 The second line of defense (modified after data from T B Tomasi and P Brandtzaeg).

of the secretory inhibitor are produced in the mucous membrane itself

(3) It has recently been proven that the high-molecular inter-alphatrypsin inhibitor in the serum can also be converted in whole or in part into a lower-molecular inhibitor by limited proteolysis In so doing, it, together with its antiproteolytic activity thus becomes capable of passing through tissue and is probably passively secreted into the nasal secretion.

It is obvious that the outfit of the mucosa with these protective proteins is also of outstanding clinical interest. The results of our working group obtained hitherto are already published elsewhere.

Up to now I have mentioned only *unspecific* protective factors in nasal secretion *Let us* now turn to the protective principles dependent on the immune system. Fig. 7 shows first of all that all known immunoglobulins can be present in nasal secretion in principle, although their presence and percentage distribution in the secretion deviates considerably from that in the serum Rather than give further details I will merely summarize the following facts

IgG represents approximately 80% of all circulating antibodies It is the principal immunoglobulin in the serum and is distributed as follows 40% in serum and 60% in extracellular fluids (Mims). IgG occurs in nasal secretion under non-inflammatory conditions in concentrations as can be expected for example by transudation from the vascular system. Under inflammatory conditions however i e in the case of increased vascular permeability the IgG concentration in the mucosa and nasal secretion is elevated Under inflammatory conditions as in the case of chronic infections in the nasal region the number of IgG-producing plasma cells in the nasal mucosa is greatly increased (Brandtzaeg et al 1967 1974) IgG can react with complement and thus activate the chain reaction at C 1 or C 3

As far as the mucosa and secretion are concerned IgG goes into action relatively late after an invasion of microorganisms.

The initial phase for IgM can be shorter

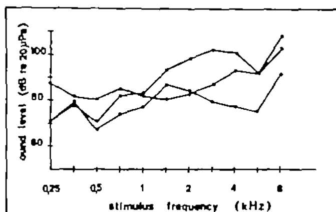


Fig 9 10 μV equal response curves for cochlear microphonic Individual results for 3 pigeons Open circles denote data from Ubbens (1955)

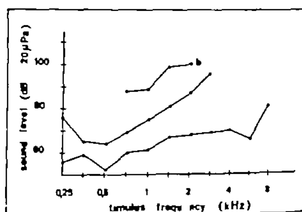
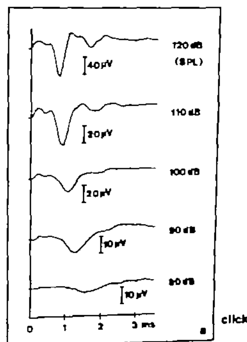


Fig 10 Average 1 μV equal response curves for microphonics (3 pigeons). *a* before cochlea extirpation, *b* after cochlea extirpation before fenestration *c* after fenestration In cases *a* and *c* 10 μV equal response curves were measured. The curves given in the figure were derived from these measurements assuming a linear relationship between sound level and microphonic amplitude.

tirpation of the cochlea in none of the animals could an action potential like signal larger than 7 μV (average of all the animals 3 μV) be detected at maximum stimulus intensity (120 dB (SPL)) One animal died shortly after the fenestration In the other animals large action potentials could again be measured after fenestration with stimuli lower than 2 000 Hz The shape of these action potentials however is rather complicated (Fig 12)

Simple-shaped action potentials could be obtained after fenestration with smoothly rising and decaying single pulses (Fig 13) The amplitude of the action potential measured after fenestration decreases faster with decreasing stimulus intensity than it does before cochlea extirpation The maximum amplitude of the action potential measured after fenestration is reached at about 0.5 ms after stimulus onset for the highest stimulus level this number increases towards about 1 ms for lower



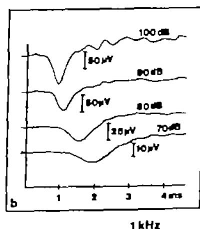


Fig 11 Compound action potential from a pigeon before cochlear extirpation In (*a*) the sound stimulus was obtained by applying one full sine wave (duration 0 1 ms) to the telephone In (*b*) the stimulus was a 1000 Hz tone burst (rectangular envelope duration 4 ms)

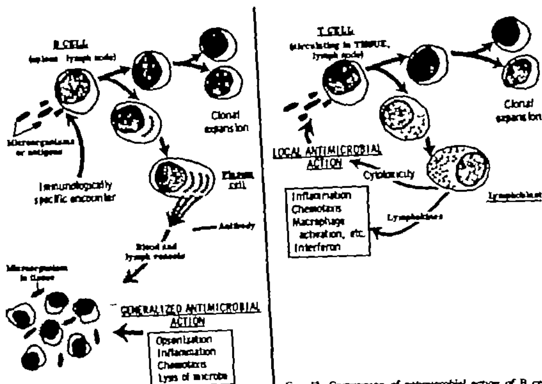


Fig. 12. Comparison of antimicrobial action of B cells and T cells (from C. A. Mims).

ders the immunocomplex in the secretion ore resistant to proteolytic activity. Secretory IgA has an antibacterial and in particular antiviral function. It can apparently activate the complement chain by the coaction of lysozyme, leukocytes and complement by way of the C3 shunt, thus triggering bacteriolysis.

Finally, IgE represents only about 0.002% of all serum immunoglobulins. It is produced in the mucosal plasma cells and can also be detected in nasal secretion, although frequently only in trace amounts. IgE becomes active as reagin in allergic reactions of the mucosa. Mast cells and basophilic monocytes have receptors for IgE, so that this immune body is capable of very quickly releasing the inflammatory mediators from the mast cells when an appropriate antigen is present.

There is so little significant information concerning IgD that one can pass on to the next point. Once a microorganism has penetrated the first line of defense represented by the secretory film, it is confronted with a predominantly mechanical barrier (Fig. 9), the formation of the epithelial layer. This barrier, however, does not appear to be an insurmountable obstacle for microorganisms, since on the one hand it is known that the epithelium can be penetrated and destroyed quickly and thoroughly—by viruses—and, on the other hand, that intravital microscopic observations have shown that even bacteria can pass through the intact epithelial aggregation including the basement membrane (Naumann 1959).

Once the pathogen has passed through the epithelial barrier as well, it is then in the lamina propria of the mucosa and thus in the second line of defense which, at the same time, constitutes the main line of resistance—tactically speaking.

Depending on the nature and aggressivity of

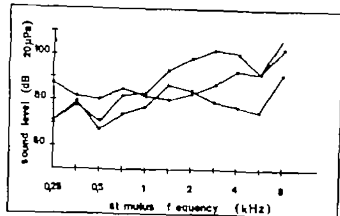


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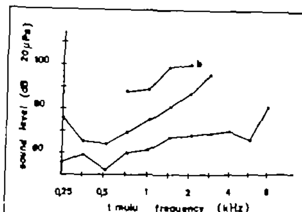
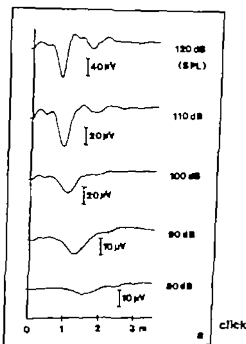


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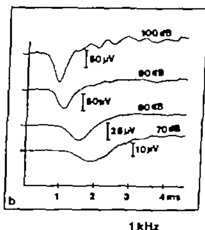


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Acta Otolaryngol 89: 177–181 1980

IMMUNOLOGIC ASPECTS OF OTITIS MEDIA

T Palva P Häyry V Raunio and J Ylikoski

Helsinki, Finland

Abstract. The cellular immune response in otitis media with effusion consisted of granulocytic cells in the majority (60–70%) of cases, while the monocytic–lymphocytic cells formed the bulk of cells present in 20%. A considerable number (15–20%) of serous, seromucinous and mucoid effusions had sparse cellular populations. T-lymphocytes, marked with α-naphthyl-acetate-esterase (ANAE) constituted the majority of lymphocytes and were present in effusion liquids in numbers similar to those in blood. Tests with E-rosettes confirmed the results obtained with ANAE stain. The finding that T-lymphocytes are present in normal numbers may link the mucosal damage to the delayed-type hypersensitivity reaction. Another explanation of mucosal damage may be sought in immune complexes, which have recently been found to be present in many sterile middle ear liquids.

The mechanism of the immunological defence against infectious middle ear diseases has been the subject of intensive studies during the last decade. It has been established that the middle ear mucosa is capable of reacting to bacterial or viral attacks in much the same way as the respiratory mucosa. Secretory otitis media, which has become the most frequent form of middle ear infection in childhood may well serve as a model for histological and immunochemical studies on the local defence mechanism.

Immunochemical Response

In 1953 Lahikainen had already shown that middle ear exudates with the exception of liquids deriving from ears in which the infection was caused by *Hemophilus influenzae* had a distinct bacteriostatic effect on the bacteria generally responsible for infection. Lysozyme, the agent thought to be responsible for this effect, is secreted by the middle ear epithelium, and is also liberated from the polymorphonuclear leukocytes and macrophages (Lim et al 1976). Lysozyme as such is a relatively weak agent against bacteria but it can act synergistically with the complement and specific antibodies. In fact, in Lahikainen's studies (1953) heating of the middle ear liquid to 60°C for 30 min to destroy the complement also deprived the middle ear liquid of its bacteriostatic effect. The lysozyme levels in the effusion have been found to be greatly elevated in mucoid secretory otitis media as compared with lysozyme levels in serum (Lim et al 1975, Virtanen & Lahikainen 1979). Similarly the immunoglobulins IgA and IgG show high effusion–serum ratios, while the levels of IgE and IgM have in general been similar to those found in serum (Mogi et al 1974, Lim et al 1976).

Apart from the really rare cases of hypo- or agammaglobulinemia, the majority of chronic secretory otitis media cases show a normal immune response. It must be admitted that there is no appreciable increase in the effused IgM over serum values, although this immunoglobulin is known to be particularly responsible for forming antibodies against bacteria. On the other hand, deficiency in IgM production has not been demonstrated. The fact that IgE values are normal proves that in the overwhelming majority of patients the immediate type allergic response is not responsible for secretory otitis media.

The explanation for this prolonged state of infection with low virulence must thus be sought in other mechanisms. In recent years two factors have been brought into focus, viz. possible changes in the T-lymphocyte re

This study was supported by a grant from the Sigrid Jusélius Foundation

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J D Bleeker MD
Kliniek voor Keel- Neus en Oorheelkunde
Academisch Ziekenhuis Groningen
Postbus 30001
9700 RB Groningen
The Netherlands



Fig. 1. Polyacrylamide gel electrophoresis of 11 middle ear effusions. The reference on the left is serum. The two runs on the right represent proteins in the Escherichia Coli capsule. Molecular weight references are on the left. The protein bands present in the effusions are seen also in the serum.

some 20–25% of specimens showed too small a number of lymphocytes to allow of a reliable estimation of their subpopulations.

For the purpose of determining the T-cell population in the peripheral blood of patients with chronic secretory otitis media, we also used the E-rosetting technique in addition to the ANAE staining. Both methods have in 10 cases given normal figures, the T-cells constituting 60–80% of the cells. E-rosette tests were also made on some cell-rich effusion specimens, generally yielding figures over 50%. However, in one effusion specimen the ANAE-positive cells amounted to only 25% and for E-rosettes the result was negative. In peripheral blood, both tests nevertheless gave values as high as 65–75%.

A few tests were also carried out with IgG anti-sheep erythrocyte antibody for demonstration of the Fc receptors. The EA-rosette formation could be seen quite frequently (50–95%) around the macrophages, and infrequently (0–15%) with the lymphocytes.

Protein Separation with Electrophoresis

41 middle ear aspirates, collected from ears having otitis media in an acute or chronic phase were subjected to polyacrylamide gel electrophoresis. Serum and liquids containing proteins of known molecular weight, were used for identification of various protein bands. Liquids containing bacterial capsular antigens with very many sharp protein bands were also employed to ensure a technically faultless separation. An example of such a run is shown in Fig. 1. When a gel with small pores was used, much of the mucous material remained at the start. With a gel with larger pores (fig. 2), the albumin was seen to migrate further towards the anode end but the mucoid material, nevertheless, remained at the start. The fastest band, present in most middle ear sera, but absent from all control liquids, was at first thought to be specific but on the further testing it proved to be hemoglobin, a contaminant in the aspirate. Otherwise, there were

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J D Bleeker M.D
Kliniek voor Keel Neus en Oorheelkunde
Academisch Ziekenhuis Groningen
Postbus 30 001
9700 RB Groninge
The Netherlands

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antibody capable of producing a specific bond with it Under physiological conditions this immune complex will be either filtered out along the RHS channels or—even though it may remain in the organism for some time—will not only be incapable of causing a T–B interaction but even of attaching to the surface of the T cell. There will be no immunoresponse This phenomenon explains the anti-D prophylaxis and is in some cases responsible for the enhancement reaction

The sixth possibility is that the antigen with few epitopes produces a complex with an antibody and generates free epitopes. In this event the antibody links with another part of the molecule increasing the epitope count to an extent at which it can bring about a T–B interaction This will obviously result in humoral antibody production

The seventh is the very special case in which the antigen is of cell size These cells must first be decomposed into protein molecules with free epitopes Depending on the epitope count, we may have a T or a B immunoresponse

The eighth possibility is a mechanism which although experimentally verified is far from unequivocal. This is the case in which the affinity energy of the antigen is low and allows only the T to attach to the cell surface Evidently this specific phase as a process responsible for the evolution of the antibody deficiency syndrome causes the disease either by damaging the macrophages or due to the congenital anomaly of the T or B cells.

Fig 4 illustrates the development of the immunoapparatus at the cell level, following its evolution from the primordial cell to the immunoresponse These very complicated differentiation processes are obviously encumbered by innumerable error sources such as a disturbed cell development or the consequences of a lack of regulation. In the latter case we should bear in mind the proportional tasks of the helper and suppressor cells According to our present knowledge and the *experimentum naturae* the two extreme states viz. complete T vs complete B deficiency provide absolute justification for the existence

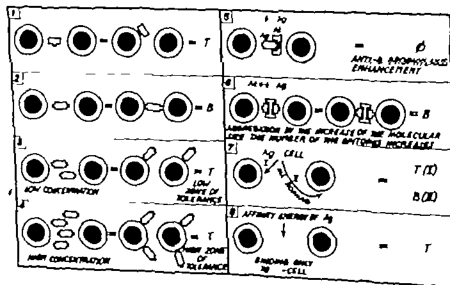


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J D Bleeker M.D
Kliniek voor Keel Neus en Oorheelkunde
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9700 RB Groningen
The Netherlands

e differs in any way from that of the individual protein is harmful and to maintain the integrity of the patient its elimination is absolutely indicated In a cellular immunoresponse a cell-cell interaction takes place which in plain terms means that the organism has little chance of taking up a fight against a large number of tumour cells. This requires an blastic operation with the simultaneous activation of the immunoapparatus so as to make it specific—bearing in mind the attempts with a so-called immunotherapy

Humoral immunoresponse is enhanced by immunoglobulins produced by plasma cells Different types of immunoglobulins perform different tasks Some of them are capable of linking the so-called K cells (immunocytes unable to identify) with say tumour cells and destroy them This is a specific form of the positive interaction of the humoral and cellular systems There is also a negative interaction when by their very presence the humoral antibodies suspend the cytotoxic effect of the cellular immunosystem for a shorter or longer period of time and bring forth the so-called enhancement reaction The mere fact that a molecule attaches to molecule, and antigen to antibody is sufficient to eliminate the harmful effect of antigen However the process is not always so simple partly because immune complexes tend to arise which if not eliminated rapidly may persist in the circulation and lead to so-called immune complex diseases Still more dangerous is the state of humoral immunodeficiency which in the form of congenital diseases may cause the five types of dysglobulinaemia.

Fig 7

The above are the so-called primary antibody deficiency syndromes Secondary antibody deficiency syndromes also exist.

They may be metabolic disturbances e g. congenital protein anomalies or the outcome of malignant afflictions of the immunoapparatus They may be caused by the disequilibrium of the chalon systems or the abnormal functioning of the helper and suppressor cells Catabolic diseases for instance the nephrosis syndrome or enteropathies with loss of protein may give rise to a special secondary antibody deficiency syndrome

For a successful interaction of the antibodies and cells the distance between two immunoglobulin molecules attached to the membrane should be 300 Å or less thus permitting the complement system to be activated and serinprotease to be generated Protease disrupts the cell membrane and due to the changed ion flow causes the quasi-explosion of the cell This proves that the integrity of the humoral immunosystem alone without the activity of the complement system does *not* ensure adequate protection.

Fig 8

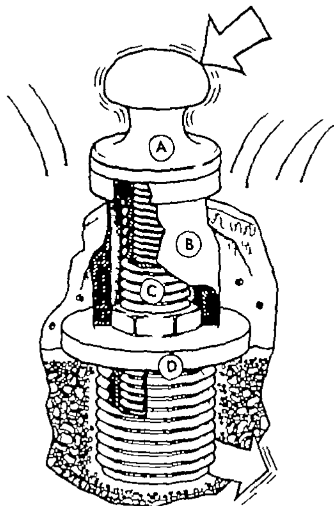


Fig 1 Osseointegrated titanium screw (A) snap fastener (B) titanium abutment, (C) connection screw (D) titanium *fixture*

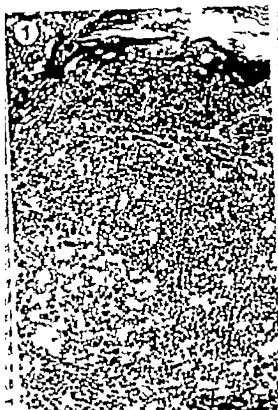
as *women who wish to have a more extreme* hair style Thus there is a need to develop a bone conduction hearing aid which minimizes these drawbacks Our idea has been to use a bone anchored fixture with a skin penetration to attach the hearing aid to This arrangement could make it possible to construct a small and discrete hearing aid which would eliminate the need of pressure and also eliminate the damping effect of the soft tissue

Osseointegration

The possibility of permanently implanting a foreign material in the body has previously been limited and a permanent reaction free skin penetration has been regarded as essen

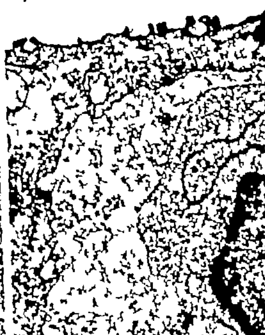
animal experiments (Brånemark & Lindströ 1963 Brånemark & Breine 1964 Brånemar et al 1969 *Hallén et al 1976*) and co responding clinical experiments (Brånemark e al 1970 Brånemark et al 1975 Brånemar et al 1977 Tjellström et al 1978*a* Tjel ström et al 1978*b*) shows that titanium ha unique *properties both in its relation to bo* tissue and to soft tissue

Anchoring of a prosthetical tissue substitut according to the principle of osseointegratio means that the surface of the implant connec directly to vital remodelling bone tissue with out any intermediary connective tissue sheat This *bio-mechanical connection is obtained* b using a combination of careful surgical tec nique based on biological knowledge abo optimization of bone healing and by the us of an inert implant material Nonalloyed tit nium is preferred the surface of which has special microstructure with an architectu and *dimension adjusted to the* cellular an interstitial components of the bone tissue Th surface of the implant as well as the bone si are protected against all kinds of contamin tion An osseointegrated implant can b supplied with extension elements for penetra ing skin and mucous membrane which will no be sites for inflammatory processes if the int gration is preserved Experience of these con *ditions have been collected for more* than 1 years During these years more than 1 500 fi tures have been implanted in the oral cavit to be used for dental rehabilitation In a pil study of three patients and a follow up stud of 11 more patients titanium fixtures hav been screwed into the bone of the linea tempo ralis of the temporal bone All these patient used conventional bone conducting hearin aids *previously* and were not satisfied wit them After the titanium fixture had integrate *with the bone for three to four months* a skin penetration was made using titanium abut ments *held in place by* titanium screws which also served as connections for the hearing aids as shown in Fig 1 The longest observa- ... 26 months and the shortest six


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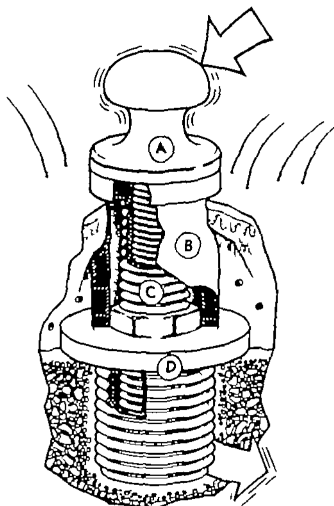


Fig 1 Osseointegrated titanium screw (A) snap fastener (B) titanium abutment (C) connection screw (D) titanium fixture

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Osseointegration

The possibility of permanently implanting a foreign material in the body has previously been limited and a permanent, reaction free skin penetration has been regarded as essentially impossible to realize Experience from animal experiments (Brånemark & Lindström 1963 Brånemark & Breine 1964 Brånemark et al 1969 Hallén et al 1976) and corresponding clinical experiments (Brånemark et al 1970 Brånemark et al 1975 Brånemark et al 1977 Tjellström et al 1978*a* Tjellström et al 1978*b*) shows that titanium has unique properties both in its relation to bone tissue and to soft tissue

Anchoring of a prosthetical tissue substitute according to the principle of osseointegration *means that the surface of the implant connects* directly to vital remodelling bone tissue without any intermediary connective tissue sheath This bio-mechanical connection is obtained by using a combination of careful surgical technique based on biological knowledge about optimization of bone healing and by the use of an inert implant material Nonalloyed titanium is preferred the surface of which has a special microstructure with an architecture and dimension adjusted to the cellular and interstitial components of the bone tissue The surface of the implant as well as the bone site are protected against all kinds of contamination An osseointegrated implant can be supplied with extension elements for penetrating skin and mucous membrane which will not be sites for inflammatory processes if the integration is preserved Experience of these conditions have been collected for more than 10 years During these years more than 1500 fixtures have been implanted in the oral cavity to be used for dental rehabilitation In a pilot study of three patients and a follow up study of 11 more patients titanium fixtures have been screwed into the bone of the linea temporalis of the temporal bone All these patients used conventional bone conducting hearing aids previously and were not satisfied with them After the titanium fixture had integrated with the bone for three to four months a skin penetration was made using titanium abutments held in place by titanium screws which also served as connections for the hearing aids as shown in Fig 1 The longest observation time is 26 months and the shortest six

minishes Due to these changes the possibility of a new infection increases and every infection results in further loss of M cells The newly formed squamous stratified epithelium is not suitable for antigen uptake but living bacteria can penetrate through it to the underlying lymphoid tissue which has fewer Ig-producing cells to defend itself One can hardly imagine other methods to stop this circulus vitiosus than tonsillectomy but further work is required to determine whether these changes are reversible or not

ACKNOWLEDGEMENT

This work was supported by the Hungarian Ministry of Health (grant no 5-07-0304-01 1/J)

RÉSUMÉ

En cas des inflammation repetées des amygdales humaines l'épithelium reticulaire se transforme sur la plupart des surfaces des cryptes en épithelium stratifié et les cellules particulières /cellules M/ sont pratiquement absentes. Les cellules M persistantes renforment moins de vésicules de transport contre les cellules analogues normaux. Dans les amygdales humaines montrantes des signes de l'inflammation répetée le nombre de lymphocytes activées est diminué

ZUSAMMENFASSUNG

Die Verminderung der Retikulierung des Kryptenepithel ist eine Antwort des Tonsillengewebes auf entzündliche Reize Die Zahl der antigenrezeptorischen Epithelzellen (M-Zellen) ist also verringert. Das Zytoplasma der restlichen M-Zellen enthält nur wenige Pinozytosebläschen Die Zahl der aktivierten Lymphozyten ist in entzündlichen menschlichen Gaumenmandeln noch vermindert.

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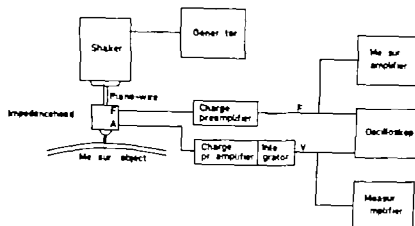


Fig 4 Block diagram of the measurement apparatus

The complex impedance Z is measured as magnitude (mechanical ohms) and phase (de grees) Calibration was performed with a known mass at 100 Hz The measurements were carried out with a vibration speed which was neither too low to detect nor too large to be unpleasant for the patients The accuracy of the electrical measurements is estimated to be ± 5% for the magnitude and ± 2° for the phase Because the system is approximately linear (Flottorp & Solberg 1976) the imped ance is insensitive to the applied signal strength

Measurements have been carried out on eight patients five women and three men Their ages varied between 30 and 60 years In order to simulate the attachment of the hearing aid to the snap fastener the vibrator and im pedance head were suspended from a whee and balanced with a weight as shown in Fig 5 The patient lay horizontally and had a sof pillow under the head in order to avoid cou pling to the supporting structures To find ou what parts of the impedance corresponded to the skull Z and to the snap fastener Z_{sf} separate measurements were made on the snap fastener according to Fig 6 The female element was fastened to a large mass 63 kg as shown in Fig 6 in order to avoid coupling to the supporting structure

The measurements were carried out at dis crete frequencies between 250 Hz and 8 000 Hz Measurements under 250 Hz are not con sidered because copuling to the supporting structures can affect the measurement result adversely

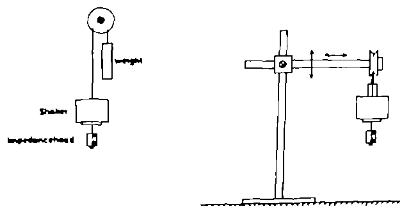


Fig 5 Vibrator and impedance head mounted in a holder which is adjustable both vertically and horizontally

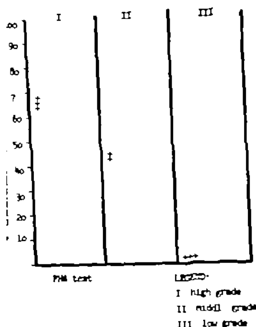


Fig. 2. PHA test and differentiation of the laryngeal cancer

phocytes are stationary and produce through the plasma cells the humoral antibodies—IgG IgA IgM IgD and IgE and IgND (as described by Johansson & Banic in 1967) The latter has been shown to have a cytotoxic effect on tumor cells. It is also supposed that IgM has cytotoxic properties with the help of a complement It is assumed that some immunoglobulins can enhance tumor growth by coating tumor cells and preventing the killing effect of T lymphocytes and macrophages

Precursors of T cells migrate from the bone marrow to the thymus where they proliferate and mature into T lymphocytes These cells circulate in the blood protecting the organism against foreign antigens They are responsible for graft rejection the defense against intracellular microorganisms and the recognition and destruction of tumor cells. While the reactive substances in humoral responses are histamines, serotonin acetylcholine and SRS (slow reacting substance) the transfer factor lymphokines are released in delayed hypersensitivity after the response of the T lymphocytes These factors as for example the chemotactic factor (chemotaxin) the migration inhibition factor (MIF) leukocyte inhibition factor (LIF) macrophage activation factor (MAF) the blastogenic factor (blastogen) and cytotoxin engage new cells in the reaction All of this amplifies the initial reaction by T lymphocytes and makes it more effective in the destruction of tumor cells.

Although T and B cells have their own radius of activity there is still cooperation between them. Among the T cells, there are those that help (helper cells) to promote the lymphocyte reaction, but also those that can suppress (suppressor cells) the immune reaction. Likewise alpha globulins have a suppressive effect on T cells and it is considered that the multiplication often prevents immunosuppression.

Our trials conducted in 1959 on laryngeal cancer revealed that immunoglobulins were elevated in the majority of cases and that their increase paralleled the growth of the tumor The opposite was found to be true for the circulating lymphocytes, which were few in advanced stages of the disease

Riesco described a positive correlation between the number of peripheral lymphocytes and the incidence of tumor cure and a negative correlation between the number of peripheral neutrophils and the incidence of cures T cells can be detected quantitatively by rosette formations and qualitatively by the blastic formation test or the DNCB test.

When correlating PHA percentages and the absolute number of T lymphocytes in laryngeal hypopharyngeal and skin cancer values were 50% or more below normal in laryngeal and hypopharyngeal cancer and normal in skin cancer (Fig. 1) There was also a positive correlation between the maturity of the tumor and PHA values Out of the 12 cases of laryngeal cancer in Grade 1 the PHA findings were normal in 7 patients and distinctly low in all 6 of the cases in Grade III

In 53 patients who underwent surgery for laryngeal cancer between 1973 and 1977 ...

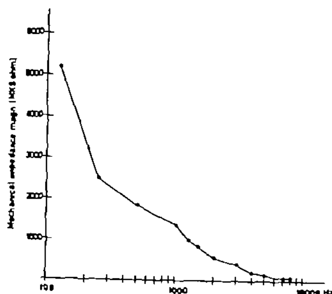


Fig 10 Mechanical impedance of the snap fastener

vidual variations and poorer precision in registering the results The results of measurements on the snap fastener are shown in Fig 10 A scattering caused by the tightness of the snap fastener (tight, medium loose) was also noted That scattering is about ±15% for frequencies below 800 Hz and very small for higher frequencies

DISCUSSION

The scattering in the measurement results is mainly explained by insufficient precision in the instruments errors in reading the instruments and varying tightness in the snap fastener used Variations in different skulls regarding elasticity and weight could also influence the scatter (Kylén 1977)

The measurement results are described in an electrical analog model in which the block elements in Figs 2 and 3 are replaced by *R C* and *L* combinations The results of the measurements on the snap fastener give a model according to Fig 11 The resonant frequency is then 7 5 kHz This means that the inductance can be neglected for frequencies below 7.5 kHz A comparison between the impedance of the snap fastener Z_{02} and that of the head shows that the impedance of the snap fastener determines the total impedence for frequencies over 2 to 3 5 kHz. This means that at higher frequencies the impedance of the skull is considerably greater than than of the snap fastener

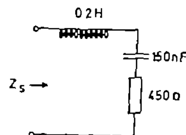


Fig 12 Electrical analog model of the skull impedance for frequencies over 2 kHz.

For frequencies below 2 to 3.5 kHz one finds that the total impedance Z_t has a resonant frequency at about 800 Hz. According to Kirikae (1958) the skull has its lowest resonant frequency between 1 7 and 1 9 kHz Kirikae s measurements were carried out on an unattached skull The resonant frequency found in our measurements at about 800 Hz can be due to the attachment to the neck and spinal cord An electrical analog of the impedance of the skull Z is shown in Fig. 12. This model is not valid for frequencies over ?

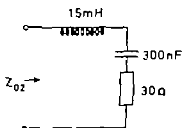


Fig 11 Electrical analog model of the snap f stener

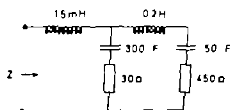


Fig 13 Electrical analog model of the combined system snap fastener and skull

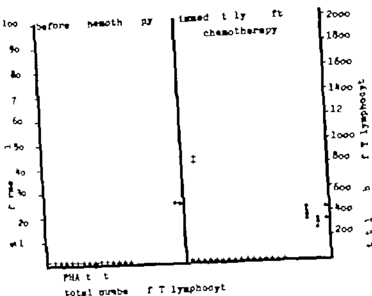


Fig. 5. PHA test and total number of T-lymphocytes following chemotherapy.

(1976) reported similar results. Therefore in addition to evaluating the patient's cellular immunity more attention must be focused on the local lymphocytic reaction around the tumor.

THERAPY AND THE IMMUNOLOGICAL RESPONSE

We wanted to investigate how the immunologic responses of the organism with laryngeal cancer change during various kinds of treatment (surgery, irradiation and chemotherapy) and thus followed the values of the PHA test and the absolute number of lymphocytes. In 21 patients with laryngeal cancer (10 with partial laryngectomy, 3 with partial laryngectomy and RND, 5 with total laryngectomy and 3 with total laryngectomy and RND) we checked these values before and 4–7 days after surgery and could not con-

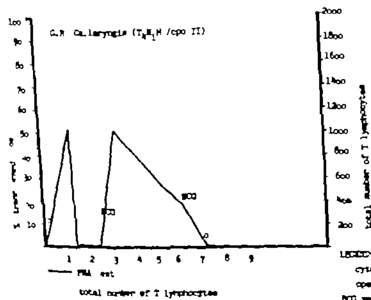


Fig. 6. PHA test and total number of T-lymphocytes in patient following surgery, irradiation and chemotherapy.

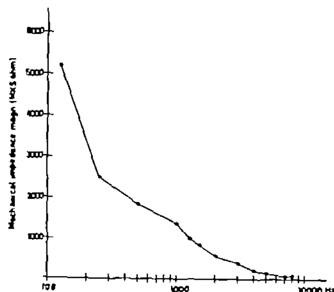


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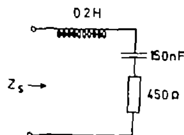


Fig 12 Electrical analog model of the skull impedance for frequencies over kHz.

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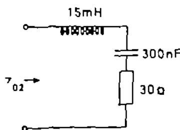


Fig 11 Electrical analog model of the snap fastener

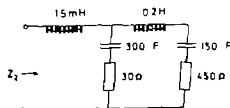


Fig 13 Electrical analog model of the combined system snap fastener and skull

CONCLUDING REMARKS

L. Surján

We have listened to some very interesting papers dealing with the oto-laryngological aspects of immunology and of inflammatory reactions I am sure than there will be an increase in interest in immunology inspired by these interesting and informative lectures

I should like to add a few comments

(1) In oto-laryngology one important problem occurring is the case of acute inflammation It seems to be forgotten that *ubi pus ibi evacua* "Where there is pus it should be drained This old Greek saying remains valid in our days too It can be observed that myringotomy has been avoided and the treatment of acute otitis media is based solely on antibiotics.

(2) It is not enough to give antibiotics against the infections—it is also necessary to take into consideration the reactions of the body and to help the defence mechanism. I believe that if the therapeutical treatment of acute inflammations of middle ear as adopted nowadays were better than the earlier one we would not observe so many cases of chronic otitis media as occurred before the antibiotic era And it is also a fact that a definite increase is observable in the numbers of adhesive processes and tympanosclerosis which originate from an earlier inflammation

(3) It seems that there is controversy regarding the timing of antibiotic application. The optimal time for commencing this treatment is not determined and the long-term results have not been investigated

(4) The real effect of antibiotics given preventively is also questionable We do not know what kind of body reactions occur in these cases!

(5) Finally I should like to suggest that we oto-laryngologists ought to think not only about the technical problems and about antibiotic treatment but also to bear in mind the defensive immunological and inflammatory reactions of the body

he found that a large amount of sound energy is absorbed by the skin (Békésy 1960) The same findings have been noted by Kylén (1977) in his amplitude analysis

Psychoacoustical aspects

Some initial psychoacoustical measurements have been performed but the results are still preliminary The patients who have received bone-anchored hearing aids emphasize that the loudness increases greatly and that the volyme control on their apparaturs must be turned down to a minimum in order to get an agreeable sound level They further emphasize that the frequency range is significantly increased in comparison with conventional bone transmission These two factors mean that the quality of the sound reaching the cochlea can be improved Much less power will be required to drive a hearing aid using an osseointegrated titanium fixture At present the hearing aid fitting is being optimized for this new situation

ZUSAMMENFASSUNG

Einige auf Hörgeräte angewiesene Patienten könne nicht einen Apparat, der den Schall durch den äußeren Gehorgang vermittelt benutzen, sondern müssen ein knochenleitendes Hörgerät anwenden. Das Gerät ist mit Hilfe eines Stahlbügels gegen die Haut des Processus mastoideus gedrückt und der Schall ist durch die Weichteile und Knochengewebe zu der Schnecke vermittelt Der Druck der das Hörgerat verursacht ist für den Patienten unangenehm. Die dampfende Wirkung der Weichteile verringert die übermittelte Schallqualität Die Moglichkeit fremdes Material reaktionslos zu implantieren um eine permanente Hautpenetration duchführen zu konnen haben es ermöglicht ei knochenverankertes Horgerat herzustellen. Vierzehn Patienten sind mit solchen Apparten versehen Wegen des neuen Verfahrens muß ein neues Gerat hergestellt werden Die Impedanz der Knochenverankerten Titanumschraube/Schädel wurde untersucht Die Resistenz und Reaktanz der mechanischen Impedanz wurden gemessen Die Einwirkung der dampfenden Weichteile wurde analysiert Der Unterschied zwischen der Impedanz des Schadels und die der Weichteile + Schadels betrung abhängig von der Frequenz, etwa 10–25 dB

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A Tjellst om M.D
ENT-Dept S hlgren Hospital
U iversity of Goteborg
S-41345 Goteborg
S ed n



Fig. 3. Electron micrograph of serous gland showing lumen (*L*), lined with few microvilli (*Mv*), intercellular canaliculi (*IC*), finger-like cytoplasmic processes (*Pr*), many desmosomes (*D*) and terminal bars (*T*). The cells contain fewer free ribosomes (*R*), granular endoplasmic reticulum (*GER*), small mitochondria (*Mi*), Golgi apparatus (*G*) and nuclei (*N*). 9625

scattered between the granules. No intact zymogen granules were noted in the lumina of the acini. There was diminished activity of the cell organelles in the form of fewer free ribosomes, smaller mitochondria and Golgi apparatus and fewer dilated cisternae of the granular endoplasmic reticulum.

In mucous cells (Fig. 4) the mucus droplets were abundant almost completely filling the supranuclear cytoplasm with marked propensity to fuse laterally with neighbouring droplets producing relatively large irregular membrane-bound aggregates of mucus. They appeared paler and of nearly the same electron density. Only a few schattered organelles were evident in the interstices between the closely

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A Tjellstrom M D
ENT Dept Sahlgren Hospit l
Univ rsity of Goteborg
S-41345 Goteborg
S eden



Fig. 7. Electron micrograph of an arteriole showing lumen (L), endothelial cells (E), endothelial basement membrane (Ebm), muscle cells (MC) and elastic fibrils (EL) in the tunica media. ×5775.

periglandular capillaries and they narrowed down as they approached the lumen. This diminished secretory activity is consequent to lesser exposure to infection and lesser need for defensive mechanism.

The preponderance of the goblet cells in the epithelium is a balancing mechanism to compensate for the diminished number and activity of the mucous acini, both of which secrete the same mucigen. This provides the mucous blanket covering the epithelial surface which is necessary to maintain ciliary function.

The diminution in the number of cilia, the delicacy and loosening of the epithelium, the diminished activity of the seromucinous glands with diminished production of bactericidal lysozyme indicate a lower resistance of the sinus mucosa to infection and easier penetration for micro-organisms.

There is a diminished exchange between tis

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A Tjellstrom M D
ENT Dept S hlg en Ho pit l
U iversity of Goteborg
S-41345 Goteborg
S ede

s 1975) shown that cell-to-cell junctions in e epithelium normally constitute a tight bar er for molecules of such a size

The second mechanism foresaw the passage rough the epithelial cell by a process of eso- ndocytosis It was later shown by South et al 1966) that this is the probable mechanism of ransport for IgA that secretory IgA differ rom serum IgA in which a protein is supplied o them during the transport through the pithelial cell and this is called "transport iece" The function of this piece is together with the J component to facilitate the trans- port and allow for the successive polymeriza tion of IgA (Tomasi, 1972)

The purpose of this work is to provide *direct evidence* of this second mechanism and to evi- dence the role of cellular organelles involved in this secretory process so as to suggest a description of the integrated sequence of cellu- lar events connected to it by means of elec tron immunomicroscopy of human nasal mu- cosa.

MATERIALS AND METHODS

(*a*) *Use of immunoenzymatic techniques in electron microscopy* They are employed to localize specifically a cellular product or com- ponent by the relative antibody conjugated with an enzyme as a marker The reaction product of this enzyme with relevant en- dogenous or exogenous substrate produces electron-dense formations which are therefore clearly visible at the electron microscope (Wil- liams, 1977*a*)

To identify the localisation of IgA in the nasal mucosa we have used complexes formed by anti-human IgA antibodies or (as a control) anti-human IgG antibodies (DACO USA) con- jugated with Horse Radish Peroxidase (HRP Sigma) using glutaraldehyde as a cross-linker according to the two-step method devised by Avrameas and described by Williams (1977*a*)

(*b*) *Procedures for the immunoenzymatic technique and for E. M* Biopsies of normal human nasal mucosa taken from the middle turbinate have been cut into thin slices and immersed into 2% glutaraldehyde in phos- phate buffer 0 1 M pH 7.3 with $CaCl_2$ for about 10 mm and then transferred into a solu- tion containing 1 mg/ml of IgG-anti-IgA+HRP conjugate or IgG-anti-IgG+HRP (con- trol) or only buffer (control for endogenous peroxidase) and incubated for 20–30 mm with continuous gentle agitation

The specimens were washed and incubated for one hour in a 1% Diaminobenzidine (DAB) solution at room temperature according to the technique suggested by Graham & Karnovsky (1966) and described by Williams (1977*a*) This step was necessary to carry out the reac tion between the enzyme (HRP) and the sub- strate (DAB) Because the reaction product is highly osmophilic, the following 2 hours post fixation on 1.33% osmium tetroxide gave the osmium-black products easily identifiable at the E. M in unstained sections

The post-fixed specimens were washed in the same buffer dehydrated in ethanol and embedded in Epon-812 Ultrathin sections were observed without any staining with a Philips 300 electron microscope

(*c*) *Quantitative evaluation* The sporadic presence of endogenous peroxidase and there by of the reaction products has made a semi- quantitative evaluation of the enzymatic reac tion products necessary We have followed the techniques and the recommendations by Weibel (1968) described by Williams (1977*b*)

RESULTS AND CONCLUSIONS

The osmium-black reaction product has been found specifically abundant in all epithelial cells of nasal mucosa. In particular the Golgi apparatus showed a highly electrondense material both in the cisternae of dictosomes and related vesicles (Fig. 1).

The large secretory granules (Fig. 2) had osmium -black product localized at the periphery near the limiting membrane The small granules and primary or secondary lyso-

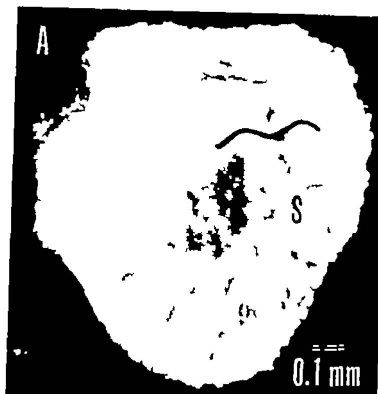


Fig 1 Normal otoconial membranes *A* the macula utriculi *B* macula sacculi right ear The curved line in *A* indicates the shape of an imaginary cross section of the *snowdrift line* (*S*) which consists of a single ridge (*S*) in the saccule. OsO

(1973*a*) has postulated that the so-called dark cells in the wall of the utricle are capable of removing calcium from otoconia which have become attached to them

During the last decade there has been a growing interest in the pathology of otoconia Abnormalities of otoconia are usually associated with a marked reduction of the crystalline layer of the otoconial membrane and an unusual size and shape of the remaining crystals The most common form of otoconial pathology in man is the marked loss of otoconia occurring with aging The loss is much more severe in the saccule than in the utricle to the extent that the macula sacculi may completely lack otoconia in older patients (Johnsson & Hawkins 1972*a b* Ross et al 1976)

Johnsson & Hawkins (1979) have described otoconial defects in man where the integrity of the vestibular epithelium or membranous wall was severely disturbed Under the influence of head trauma or ear disease otoconia may become dislodged from the utricular macula and enter the posterior ampulla provoking a disorder which Schuknecht (1974) has termed cupulolithiasis

Complete absence of otoconia from all four vestibular maculae in a 6-week-old infant was recently reported by Wright Hubbard & Graham (1979*b*) This anomaly which apparently was congenital in origin was strikingly similar to that present in certain genetically defective animals such as the pallid mouse in which otoconia fail to develop (Lyon 1951) Otoconial defects are now known to occur in at least five different mutant rodents and in one species of partridge (Lim & Erway 1974) Also abnormal otoconia have been observed in both the saccule and utricle of the deaf Dalmatian dog (Johnsson et al 1973) which like the mutant mice suffers from a genetically determined pigmentation defect

Acid solutions and other decalcification agents can obviously alter or dissolve the otoconia which consist of calcite i e calcium carbonate crystallized in trigonal form The otoconial membranes are also very sensitive to mechanical trauma The membranes be-



Fig. 3. Detail of the above picture. The smallest secretion granules with homogeneous content do not contain the reaction product. On the contrary, the osmium-black reaction product is well visible at the periphery of the large granule with dense and homogeneous content. Unstained section × 60 000



Fig 2 Abnormal-appearing pair of otoconial membranes from the right ear of two untreated normal pigmented guinea pigs *U* macula utriculi *S* macula sacculi A mild (*top*) and a more severe (*bottom*) degree of reduction of otoconia in the crystalline layer of the otoconial membrane The apparent loss of otoconia is more diffuse and severe in the saccule (*lower right*) while it is clearly concentrated along the striola in the utricle Numerous otoconia are scattered in the saccule OsO_4

concentrations of alcohol (35% 50% 70%) The largest number of specimens was dissected 2–4 days after fixation but particular care was taken to dissect several specimens on the day of sacrifice in order to confirm the presence of abnormal findings prior to any lengthy storage in alcohol The examination of the otoconia was done primarily with the dissection microscope A few otoconial membranes were studied as surface preparations in phase-contrast and dark field illumination The technique of microdissection used in this study is described in detail elsewhere (Hawkins & Johnsson 1976)

MATERIAL

Thirty two colored guinea pigs which had received no drugs served as the normal material Furthermore two series of 75 and 53 albino guinea pigs were examined Several of these animals had been treated with streptomycin or neomycin An additional series of 8 normal albino animals were also studied T

RESUMÉ

r rôle des anticorps secrétoires IgA dans la défense ntivirale et antibactérienne des hautes voies aériennes é amplement démontrée et al détermination des IgA ns la sécrétion nasale est devenue à notre avis, de la us grande importance dans l'étude clinique rhinologique. Le mechanisme par lequel l'IgA arrive à la secrétion sale à travers la muqueuse est encore objet de discussion aucune des deux hypothèses de Tomasi (libre filtration travers l'interstice epithelio-connectival ou transport ntracellulaire avec secrétion active) en jusqu'ici de monstration véritable.

En employant une IgG anti IgA humaine purifiée arquée par de la peroxydase de horseradish, nous avons ydié d'une façon semiquantitative sur des sections mi-fines fines, le comportement de l'IgA au niveau de muqueuse nasale humaine

On pu demontrer que l'IgA elaborée et secrétée dans nterstice connectival par les cellules plasmatiques speci-ques, serait incorporée par endocytose par les cellules ithéliales, ou elle est visible surtout dans les corps de olgi et dans les grandes granulations de secretion ique dans le transport endocellulaire de vesicules de tocytose un rôle est étément joué par les microfilaments ctine et par les microtubules, avec utilization de ATP serait intéressant d'investiguer en employant soit des stems physiques (tels que le froid) qui empêchent l'utili-tion de l'ATP soit des agents chimiques (tels que la tochalasine et la colchicine) qui dépolymérisent respec -ement les microfilaments et les microtubules, si l'on ut obtenir des epreuves ultérieures en faveur du mé-anisme de transport actif de l'IgA suggéré par les re-ltats de nos études présentes

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S Crifo M.D
Dept Otolar and Audiology
Polic m Umberto
I-00100 Roma
Italy

DISCUSSION

Surjan J to Crif You have presented nice description of IgA transport and modern sensitive method was applied The result is similar to that of Brandtzaeg. The presence of IgA in the Golgi of epithelial cells is, however surprising. I this respect please let me know how was your anti-IgA purified and how is the specificity proved?

Crifo (Reply)

Thanks to Dr Surjan Jr for his comment. The sensibility of the electron-immunomicroscopy is more valuable than the immunofluorescent method. Such technique permits study at the level of endocellular organelles in this way is possible the reconstruction of the dynamic events occur ring during or after given phenomenon. I would be grateful for any advice giving me notice or previous documentation of IgA-active transport at the level of the mucous membranes. Naturally all people retain today this mechanism more probable but w have not found in the literature any documentation on it, similar to that which have reported.



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		Test results recorded by Lab Technician A					
		–	+	+	+++	++++	Total
st results corded by b Techni- n 'B'		814	54	18	1	0	887
		54	29	20	7	0	110
	+	26	27	22	4	0	79
		6	7	11	18	2	44
	+	0	0	1	4	2	7
	Total	900	117	72	34	4	1 127

re added to the heated serum before incuba on with food antigens

(e) To block Ca^{2} and Mg^{2} ions one drop of EDTA (ethylendiamine-tetraacetic acid) distilled water was added to 12 drops of rum and the buffy coat leukocytes This sol ion was used in place of the distilled water rmally used in cytotoxic tests

(d) To inhibit the possible histamine release om the leukocytes one drop of 1 5 10 and 0% DSCG (disodium cromoglycate) solution as added to the leukocyte–food antigen pre aration.

( ) To block possible H -receptors on the ells one drop of antihistamine solution Phenergan[a] 25 mg/ml Tavegyl[a] 1 mg/ml) as added to the cell suspension

(f) The effect of steroids on the cytotoxic eaction was tudied by substituting cortisone ompound solution (Betnesol[a] 1 25 000 olu-Cortef[a] 1 000 1 200) for the drop of istilled water in the cytotoxic tests

4 Histamine chloride and histamine acid hosphate were used in concentrations of 1 1000 1 100 1 10 and 1 5 in order to study he effect of histamine on leukocyte pseudopod formation. The solutions were made from the powder in sterile distilled water and substituted for the distilled water used in the cytotoxic test

RESULTS

1 Identical results were recorded in 78% of 1 127 tests read double-blind by two labora torytechnicians A slight divergence in regard to the severity degree of the reactions oc curred in 16% and a greater deviation in 6% The statistical error of the method was 0 46 (*Table I*)

2 In the series of tests repeated on 3 consecutive days 81% of the results recorded for the first day agreed with those noted for the second day 80% of the test readings for the first and second days showed agreement and 82% of the tests second-day and third-day results were concordant The statistical errors were 0 41 0 43 and 0.38 respectively

3 In 42 of 58 tests (72%) a positive leukocyte reaction was transferred with the reactive serum to cells from non-reactive subjects. By

Table II *The effect of heating (56°C 2 h) on the cytotoxic reaction*

	Number of positive reactions	Per cent
Original cytotoxic test	57	100
Heated serum unwashed cells	4	46
Heated serum washed cells	11	1

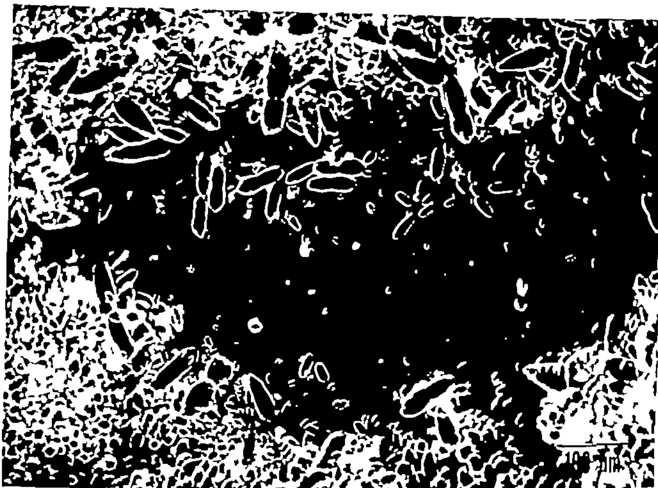


Fig 4 Detail of a defect similar to that shown in Fig. 3 Several large dumb-bell shaped otoconia, up to 90 μm in length are seen

conial layer extended over the whole surface of the macular neuro-epithelium and 2) animals in which the otoconia covered only a part of the neuro-epithelium The otoconial membranes of Group 1 were rated as normal' those of Group 2 as abnormal' because the latter deviated so markedly from all previous descriptions of the macular organs in normal mammals (Figs 1–5)

Normal otoconial membranes

There is little new information in the present study to be added to the many descriptions of normal otoconial membranes in guinea pigs (Fig 1) The line of the snowdrift on the saccular otoconial membrane resembles that seen in man On the macula utriculi on the other hand a single relatively sharp ridge was not present as in human specimens (Johnsson & Hawkins 1967 Wright & Hubbard 1978, Wright, Hubbard & Clark 1979*a*) Instead, two low ridges were seen running in parallel to form the usual "U' shaped line Between these there was a shallow groove Lateral and posterior to this double line of snowdrift the surface was more rippled than elsewhere often forming a pattern of small ridges which tended to radiate toward the margins of the membrane more or less perpendicular to the snowdrift line (Fig 1) When the opposite side of the membrane which faces the neuro-epithelium was examined a more darkly stained curved line was seen in the gelatinous part of the membrane under the snowdrift In agreement with Lindeman s (1969) earlier observation we found that the membrane along

actions. The results are thus somewhat contradictory and it seems that the cytotoxic changes in the leukocytes must also be caused by some factor(s) other than complement activation. Whether for instance the lymphocytes play a role via liberation of lymphokines remains an open question.

Factors possibly connected with histamine release and its blocking were tested by adding DSCG and antihistamine to the leukocyte–food antigen preparation. The protection provided by DSCG may probably be attributable to the stabilizing effect which this drug exerts on the cell membrane. Antihistamines were found to inhibit expected reactions in 93% of tests. This can hardly be due to the H -receptors competing successfully on the cellular level but it is more likely that antihistamines also acted as cell membrane stabilizers. Similarly cortisone, when present in a sufficiently high concentration around the cell, seemed to have a stabilizing effect on the cell membrane. However, these in vitro results do not necessarily imply that the same effects would be produced *in vivo*.

To sum up, the cytotoxic leukocyte reaction appears to be a multifaceted process which is effected by factors as yet unknown. The reproducibility of the test is fairly good provided that the technicians are properly trained. The definite significance of the cytotoxic test in the diagnosis of food allergy is yet to be elucidated. The test usually gives negative results in so-called fixed food allergy, and its importance may well lie in the diagnosis of chronic, cyclic food allergy. However, not all positive reactions in a test can be taken to indicate clinically manifest food allergy, and provocation tests and/or elimination diets are necessary in order to determine whether the foods indicated by the test can be held responsible for symptoms.

RÉSUMÉ

T[illegible] cytotoxiques ont été faites dans 49 spécimens du sang et les réactions étudiés par deux techniciens à la manière du double-blind. Les résultats identiques ont été trouvés dans 78% des cas avec une erreure statistique de 0.46. Dans une groupe de 32 malades, le même test cytotoxique a été répété en 3 jours consécutifs. Des résultats différents ont été trouvés dans 18–20% des test avec une erreure statistique de 0.38 et 0.43. Spécimens du sérum (75 sujets) avec une réaction cytotoxique leucocytaire après un contact avec des antigens alimentaires ont été étudiés et les observations suivantes faites. Dans 42 des 58 tests la réactivité a été transférée avec un sérum positif dans les leucocytes normaux. L'échauffement à +56°C pendant 2 heures et le lavage des cellules ont éliminé la réactivité dans 44 cas du 52. Le traitement des cellules avec EDTA dans 36 spécimens complètement bloqué la réaction cytotoxique. DSCG a bloqué la réaction dans 45% les antihistamines dans 93% et la cortisone dans 20% des tests.

ZUSAMMENFASSUNG

Mit Blutproben von 49 Patienten wurden im Doppelblindversuch zytotoxische Teste ausgeführt und von zwei Laboranten gewertet. In 78% der Teste waren die Resultate identisch und der statistische Fehler der Methode war 0.46. In einer Reihe von 32 Patienten ist der zytotoxische Test an 3 aufeinanderfolgenden Tagen wiederholt worden. Die Auswertung ergab verschiedene Resultate in 18–20% und statistische Fehler zwischen 0.38 und 0.43. An Serumproben von 75 Personen mit zytotoxischen Leukozytenreaktionen auf Nahrungsmittelantigenen wurden im Anschluß an zytotoxische Teste verschiedene in-vitro-Experimente ausgeführt. Die folgenden Beobachtungen wurde gemacht. I 4 von 58 Testen wurde die Reaktivität mit dem Serum zu normalen Zellen über führt. Erhitzung de Serumproben während Stunden in einem W sserbad von 56°C und Waschen der Zellen eliminierten die Reaktivität in 44 von 52 Proben. I 36 Testen ergab die Behandlung mit EDTA ausnahmslos eine totale Blockierung der zytotoxischen Reaktion. DSCG blockierte die Reaktion in 45% Antihistamin in 93% und Kortison in 20% der Teste.

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E. Holopainen
Dept. of Otolaryngology
University of Helsinki
SF-00290 Helsinki 29
Finland

ity always involved both the saccular and utricular maculae

In the abnormal specimens the reduction of saccular otoconia was quite variable Characteristic findings are shown in Fig 2 Compared with the utricle the apparent loss of saccular otoconia was invariably more extensive The more severe the changes were in the saccule the more pronounced was the defect in the utricle In both saccule and utricle numerous otoconia were found scattered around the macula and adherent to the membranous wall although the latter phenomenon was mostly confined to the utricle We observed no otoconia in the ductus reuniens or in the cochlea Moreover there seemed to be no tendency for the otoconia to fall into the posterior ampulla

During dissection the otoconia became dislodged more easily from the defective membranes than from the normal specimens apparently because the otoconia were less adherent to the gelatinous membrane in the abnormal specimens The otoconia varied greatly in size When the reduction of saccular otoconia was severe the crystals often appeared translucent rather than milky white and some of them had a roundish shape

On the abnormal utricular maculae there was a marked reduction of otoconia in the area where the snowdrift was present in normal specimens This gave rise to a deep groove in the otoconial layer which left the gelatinous portion of the membrane uncovered and transparent The darkly stained neuroepithelium was visible through the bare parts of the membrane creating the appearance of a black curved strip above the striola The posterior half of this strip was always wider than the anterior half

As illustrated in Figs 3 and 4 single otoconia which were visible in the area of the defect were usually much larger than elsewhere on the membrane and they often had a peculiar dumb-bell shape much like the otoconia described by Lim et al (1978) in the tilted-head mutant mouse

In surface preparations the reinforced of the gelatinous layer of the otoconial m brane above the striola could clearly be (Fig 5) In general the appearance of utricular membrane was similar to that in however the saccular membrane in the gu pig lacked the striking and complicated rangement of fibrils seen in man (Johnsso Hawkins 1967)

In the animals with an incomplete set of otoconia the number of crystals attache the surface of the membraneous wall of utricle was usually greater than in nor animals In both the normal and abnor utricle the otoconia on the wall had the app ance of a thin layer of powder snow The tribution of such crystals was not rand Circumscribed white areas were seen cove areas of the wall which stained darker t the surroundings In most guinea pigs a tr verse sharp border could be discerned se rating a translucent light zone of the wall above the anterior third of the macula fr a darker posterior region A sharp line of marcation was usually present created by layer of otoconia covering the darker a (Fig 6) In the samples of utricular wall st ied by light microscopy it could be s that the otoconia which were adherent to wall were often clustered within the boun ries of single cells This finding seems to similar to what has been demonstrated by L (1973a) in a scanning electron microgra When a large number of otoconia were pr ent the distribution was more even and t characteristic pattern in Fig 6 could not seen These otoconia varied in size but mo of them were small approximately 1 µm less in length They were highly refractile a they did not appear to be in the process degeneration

In 2 animals from the series of 8 albin guinea pigs studied a single giant irregularl shaped otoconium or otolith was found o each macula (Fig 5) Only one ear was avai able from each of these animals it is therefor not known whether the anomaly was bilatera

Table I *Statistical description of rhinomanometric and radiographic measures*

\	M	S.D	Min.	Max.
23	.99	1.58	1..	7.3
4	4 83	2.91	0 0	9.0
4	8.38	3 57	0.5	15.0
4	11 48	5.25	1.0	19.5
4	4 44	.64	0 0	10.0
4	18 75	3 51	1.5	29 0
4	30.58	4 77	22.0	39.5

L Nasal respiratory resistance in cm H_2O/l/sec
P T T Radiographic measures (Fig. 1) in mm

₄ The shortest distance from the upper face of the palatine velum to the adenoid ue

. Depth of the soft tissue

₁ The soft tissue shadow on a line from pterygomaxillary point to the midpoint of line joining basion and the center of sella cica (Linder Aronson, 1970)

₂ The soft tissue shadow on a line from pterygomaxillary point to basion (Lin- Aronson 1970)

On the postero-anterior radiographs the lth of the airway was measured as the test distance between the lateral choanal rders

inomanometry

e NRR was measured with a commercially ailable rhinomanometer the Mercury Eltronics Nasal Resistance Meter NR1 e instrument was modified to permit an rior as well as posterior recordings and an tra digital display was added to permit re rding in the natural respiratory rhythm. A ethod for individual lining of the nose mask a developed for tight fitting. A biological ed-back procedure based on an oscilloscope splay of the flow-pressure diagram gave inrmation to the patient on the proper method breathing. In this way all children down to e age of 5 years could participate in the amination. For a detailed description of the ethodology see Solow & Greve (1979 1980) Posterior as well as anterior rhinomanometry was performed In the present study only the posterior recordings were used

The measurements were calculated as the mean of four series each comprising four re cordings at a constant flow threshold of 0 1 l/sec for the anterior recordings and 0.2 l/sec for the posterior recordings The method error within a session was found to be 0.2 cm H_2O/ l/sec

Before the recording Neosynephrine® 0.5% was administered as nose drops in order to eliminate variations in the NRR due to congestion of the nasal mucosal membrane

Surgical assessment of the size of the adenoids. The amount of removed tissue was given a semiquantitative evaluation recorded as − + ++ +++

RESULTS

The presurgical figures from rhinomanometry and radiography are given in Table I The most narrow passage in the nasopharyngeal airway was found at P and P All measurements of passage showed wide inter-individual variations The depth of tissue was always most pronounced at T The mean at T_1 was 18 75 mm which exceeds significantly the limit of 15 mm set by Johanneson (1968)

The radiographic measures of the nasopharyngeal airway P_r–P showed highly significant intercorrelations (r=0.83–0.95 p<0 001) and the measures P_r–P showed correlation of about −0.5 (p<0 05) with NRR (Table II Fig. 2) The amount af adenoid tis

Table II *Correlations between radiographic and rhinomanometric measures of the nasopharyngeal airway*

Radiography	Rhinomanometry NRR
P	−.52
P	.47*
P	.46
P	−.38

* p 0 05

ity always involved both the saccular and utricular maculae

In the abnormal specimens the reduction of saccular otoconia was quite variable Characteristic findings are shown in Fig 2 Compared with the utricle the apparent loss of saccular otoconia was invariably more extensive The more severe the changes were in the saccule the more pronounced was the defect in the utricle In both saccule and utricle numerous otoconia were found scattered around the macula and adherent to the membranous wall although the latter phenomenon was mostly confined to the utricle We observed no otoconia in the ductus reuniens or in the cochlea Moreover there seemed to be no tendency for the otoconia to fall into the posterior ampulla

During dissection the otoconia became dislodged more easily from the defective membranes than from the normal specimens apparently because the otoconia were less adherent to the gelatinous membrane in the abnormal specimens The otoconia varied greatly in size When the reduction of saccular otoconia was severe the crystals often appeared translucent rather than milky white and some of them had a roundish shape

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...le III *Correlations between presurgical symptoms*

	RUAI	Snoring	Rhinolalia	Mouthbreathing
...AI		19	.55	00
...ring	19	–	42	48
...lalia	.55	.4_		40
...thbreathing	00	.48	40	

...0 05

...phic measures of the nasopharyngeal air... y and NRR was found for the dimension P ...bbert & Whitehouse 1978) i.e the shor... t distance from the most anterior part of the ...noidal mass to the posterior wall of the ...xillary antrum which is usually found in ... upper part of the nasopharynx. P also ...wed higher correlations with Snoring than ...and P_3 Moreover Fig. ? shows that in the ...jects with the highest presurgical values of ...R, the predominant indication for ...noidectomy was Snoring These observa... ...ns suggest that a high location of the ...noid tissue leads to increased NRR and ...oring.

Among the symptoms considered, the best ...rrelation was found between RUAI and ...inolalia. Recurrent Upper Airway Infection ... often considered an indication for ...enoidectomy Judged by radiography how... ...er children with RUAI did not present re... ...ced size of the passage in the nasopharynx ...heir main disease thus may have been lo... ...ted in the nose a finding which suggests that ...UAI alone is not indication for adenoidec... ...my

The correlation analysis further demon... strated an association between Snoring and Mouthbreathing. Thus relates to the find ing that the highest correlation between the radiographic dimensions and Snoring was found for the dimension P The mechanism behind these relationships could be that eleva tion of the soft palate in Mouthbreathing re duces the distance P_4 between the velum and the adenoid tissue and facilitates the oscilla-tory movement of the soft palate causing Snor ing

The radiographic measures of the depth of the adenoid tissue (T_1 and T_2) and the size of the airway (especially P and P) showed rather similar correlations with impaired nasal breathing (Snoring and Mouthbreathing) This indicates that both types of measurement are of value in determination of the clinical indica-tion for adenoidectomy

The relation of tubal dysfunction to nasopharyngeal airway is outside the scope of this study According to Mawson (1968) and Rynnel-Dagöö (1978) recurrent ear inflamma-tion and secretory otitis media are not *per se* indication for adenoidectomy

The present study suggests that adenoidec tomy should be performed only in children with obstruction of the nasopharyngeal airway

...ble IV *Correlations between presurgical ...mptoms and measures of the nasopharyn-...eal airway*

...mptoms	Nasopharyngeal airway				
	NRR	P	P	P	P
...UAI	.38	03	13	1	.08
...noring	.33	– 42*	.28	36	45
...hinolalia	.26	.03	10	.06	–.00
...outhbreathing	.30	–.25	– 17	–.30	–.28

...p<0 05

Table V *Correlations between presurgical symptoms and measures of adenoid tissue*

Symptom	Adenoid tissue	
	T	T
RUAI	–.08	–.07
Snoring	40	41
Rhinolalia	.00	.05
Mouthbreathing	.31	.38

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In 2 animals from the series of 8 albino guinea pigs studied a single giant irregularly shaped otoconium or otolith was found on each macula (Fig 5) Only one ear was available from each of these animals it is therefore not known whether the anomaly was bilateral

Acta Otolaryngol 89 233–239 1980

CHOLESTEROL GRANULOMA

J Sadé A Halevy A. Klajman and T Mualem

From the Meir Hospital Kfar Saba and The Tel Aviv University School of Medicine Israel

Abstract The idiopathic haemotympanum is in reality a persistent secretory otitis media. Histologically it demonstrates chronic granular mastoiditis associated with cholesterol granuloma. This, however is not a very specific finding and may be frequently found in other types of the "underaerated middle ear syndrome" such as ears with central perforations or indeed with cholesteatoma. In these granulomas, iron deposits are found, as well as the iron-chelating agent—lactoferin, which is known to be part of the defence mechanism of all mucosa. Lactoferrin is specifically secreted by the exocrine glands and it is not surprising to find much of it in the middle ear in situations where mucus is being abundantly synthesized—as the case may be in any otitis media. There is no real clinical or histological evidence so far that neither the iron deposits nor the cholesterol crystals of these granulomas emanate from previous haemorrhage

Idiopathic haemotympanum" is a condition where the tympanic membrane is tinted blue—and is therfore also called "The Blue Ear drum On exploration these middle ears are found to contain granulomas in which cholesterol crystals as well as iron deposits can be demonstrated The purpose of this study was to see whether lactoferrin an exocrine gland-secreted iron-chelating protein is associated with these granulomas and the iron therein.

METHODS

The tissue to be examined was quick frozen on dry ice immediately after removal and stored at −80°C Later it was cut with a cryocut into 4 μm thick sections at −70°C Mucus was examined after it was smeared on glass slides The sections and smears were dried and subsequently incubated with anti-lactoferrin antiserum (DAKO Copenhagen Denmark) diluted 1 8 for 30 min at room temperature rinsed in saline phosphate buffer pH 7.2 (PBS) for 20 min incubated with fluorescein-conjugated anti IgG (DAKO) diluted 1 20 for 70 min at room temperature rinsed again in PBS for 70 min mounted in buffered glycerin covered with a coverglass and observed under ultraviolet light with a Zeiss Standard Universal microscope with epifluorescent condensor The magnification was ×400 Pictures were taken with a semi-automatic camera. The presence of lactoferrin was demonstrated by an apple-green fluorescence Negative preparations showed no fluorescence

MATERIAL

141 specimens from 51 patients were examined grouped as follows

A 10 samples of mucus, retrieved from secretory otitis media ears

B 3 biopsies from the promontorium of ears taken during tympanometry (for stapedectomy) from 3 patients

C 15 biopsies from chlesterol granulomas from 5 patients

D. 17 biopsies *from* the mucosa and granulation tissue from 4 ears with dry chronic otitis media, which came for tympanoplasty

E. 45 biopsies from the mucosa granulation tissue from 9 patients having wet thick *mucosa* in simple chronic otitis media who came for tympanoplasty

F 20 biopsies from the mucosa, granulation tissue and matrix, from 9 ears with cholesteatoma, which came for surgery

G 30 biopsies from salivary glands taken during various salivary gland surgical operations from 10 patients

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Established Investigator of the Chief Scientist's Bureau Ministry of Health, Israel Offprints requests: Jacob Sadé M D E.N T Department, Meir Hospital, Kfar Sava, Israel.

cherry red or pink) and coat color Animals with defective otoconial membranes were found in all categories of eye and coat color In our small sample it happened that the incidence was highest (4 out of 9) in the pink eyed animals with light coats (e g *yellow tan* light grey) and lowest (4 out of 17) in animals with dark eyes and dark coats A total of 10 of the 32 colored guinea pigs had otoconial defects

In the series of 75 albino guinea pigs used in the ^{45}Ca uptake study some of which were treated with neomycin and streptomycin 14 animals had abnormal otoconial membranes The overall incidence of animals rated as abnormal in the combined sample of 107 guinea pigs was 22 4% In a subsequent series of 53 animals which included both untreated as well as streptomycin and neomycin treated animals 17 animals with abnormal otoconia were found

DISCUSSION

It could be argued that what we have described as an abnormality in the vestibular system of seemingly normal animals simply represented a normal variation of the mass number distribution and structure of the otoconia As mentioned above however the otoconial membranes rated as abnormal differed so markedly in appearance from previous descriptions and from what we have observed in a variety of animals including monkeys dogs cats and several species of rodents that they evidently must represent some form of anomaly or pathology We have found similar defects of the otoconial layer in the utricle of the rat and gerbil but in no instance was the deviation from normal appearance as marked as in the guinea pig nor was the incidence nearly as high

It i noteworthy that the pathological otoconial membranes occurred in colored as well as in albino guinea pigs One can only speculate about the possible cause for the finding We do not know whether the abnormality was congenital or was acquired after birth question of whether the finding represent agenesis dysgenesis or degeneration of e ing otoconia therefore remains open con ering the relatively young age of the anim the most likely explanation for the presenc the abnormal otoconial membranes is a c genital hereditary condition perhaps resul from long term inbreeding

Because of the sparsity of otoconia in abnormal specimens numerous crystals co studied in detail but a comparison with n mal samples was difficult because the otoco were not as well displayed in the dense crys line mass of normal membranes particula in the deeper layers Therefore it could be unequivocally established that the lar unusually shaped otoconia which we obser (Figs 3–4) were actually abnormal in gui pigs Only the specimens shown in Fig 5 w analyzed by powder X ray diffraction Judgi from the shape of the otoconia we found other abnormal membranes however most the crystals probably consisted of calcite

Although systematic behavioral testing w not carried out in the present study the a mals with otoconial defects (including tho having single giant crystals on each of t maculae) exhibited no obvious postural or b havioral abnormalities With regard to this o servation it might be noted that Lim et a (1978) have shown that animals with genetica ly determined otoconial defects often displa normal air righting reflexes and swimming abil ity even though the total area of sensory ep thelium actually covered by otoconia may b much less than normal These workers postu late that even a single large crystal attache to the gelatinous otoconial membrane ma provide a uniform stimulus for a large area o the neuro-epithelium In addition the absence of overt behavioral signs might to some ex tent reflect the intervention of central nervous compensatory mechanisms brought into play in the presence of congenital defects of the peripheral receptor organs

The function if any of the accessory oto-

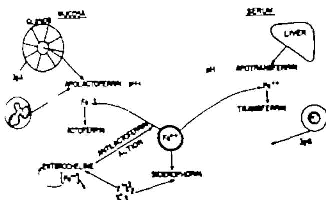


Fig 4 Diagram showing how various factors, belonging to bacteria and host, compet among themselves for available iron.

et al. 1966*a* 1966*b*) of which the middle ear by virtue of its respiratory epithelial lining is also a part (Sadé 1966) Neutrophils also produce lactoferrin (Schafer 1951) Since its discovery in 1939 by Sørensen (1939) interest in lactoferrin has grown and numerous studies have been made of it (Biserte et al 1963 Blanc & Isliker 1961 Johansson 1960 Masson et al. 1964 1966*a* 1966*b* Montreuil & Mullet, 1960 Sadé 1966 Schafer 1951) Lactoferrin is a glycoprotein to which iron ions bind reversibly with a molecular weight of 80 000 daltons (Masson et al 1966*b* Blanc & Isliker 1963 Schade et al 1949) When it is secreted by the mucosa in an iron-free form it is termed apolactoferrin. This system present in exocrine excretion, is very similar to the humeral iron-chelating system the transferrin-apotransferrin system, which is a plasma protein synthesized in the liver In analogy to the IgG and IgA system we also have here a double anti-bacterial' system—the one functioning in the circulation (transferrin and IgG) the other on mucosal surface (lactoferrin and IgA).

The antibacterial action of the iron-chelating agents is related to the dependence of bacteria and fungi on the presence of iron (Arnold et al. 1977 Hansen et al 1975 Kirkpatrick et al 1971 Reiter et al. 1975) Microorganisms are devoid of iron deposits and are therefore in constant' need of iron supplies they develop and multiply according to the amount of iron available In mammals however iron is usually not present in a free form but is protein-bound It can be retrieved by bacteria by means of their own iron-chelating agent called siderophorin which can transfer iron ions from host to parasite Apparently lactoferrin is called in to counter this activity The host–parasite competition for iron is complicated by an anti-lactoferrin agent produced by bacteria, termed enterochelin (Rosenberg & Young 1974) (Fig. 4) Bullen et al (1971 1972) also supplied evidence to suggest that lactoferrin can act synergetically with specific antibodies and provide a more powerful antimicrobial system than either component alone The production of enterochelin is increased by bacteria in the absence of iron and the capacity to produce it is consequently a factor in bacterial virulence (Kochan et al. 1977) Lactoferrin s activity is highest at a pH below 7 transferrin at pH values above 7 This prompted on of us (A H.) to suggest that some of the bacteriostatic effect of lowering the pH as performed for example in chronic middle ear infections by using boric or acetic acid could be related to this

To sum up the presence of iron deposits in a secretory mucosa, as found by us in blue ears in middle ear mucus or any other chronic and inflamed middle ear mucosa could therefore be explained as secondary to the chelating effect of lactoferrin The finding of lactoferrin in these instances is not really surprising, as lactoferrin is secreted by most mucosas—of which the middle ear is only one

ZUSAMMENFASSUNG

Die Otolithenmembran vieler albino- und normalen pigmentierten Meerschweinchen wurden mit Lichtmikroskope untersucht Es stellte sich durch das Mikrosezieren heraus daß die kristallinische Schicht der Otolithenmembrane unvollständig war denn man fand große Teile der gelatinösen Fläche unbedeckt von Kristallen Diese Defekte wurden an beiden Seiten gefunden sie waren mehr oder weniger symmetrisch und ausnahmslos stärker im Sacculus vertreten als im Utriculus In zwei Meerschweinchen wurden einzelne große Anhäufungen der Kristallen gefunden an der Macula sacculi sowie an der Macula utriculi Keines der Tiere zeigte Anzeichen von anomalem Benehmen und anomaler Haltung und man fand keinen auf der Hand liegenden Grund für die Otolithendefekte Die Anwesenheit einer vererbten angeborenen Veranlagung ist naheliegend Das häufige Vorkommen der Otolithendefekte in Meerschweinchen macht diese Tiere ungeeignet für die meisten Untersuchungen der versuchsweise eingeführten Pathologie Darum sollten im Hinblick auf die jetzt vorliegenden Untersuchungen vorherige Studien der Otolithen-Pathologie neu überprüft werden

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Lars-Göran Johnsson M.D
Kre ge Hearing Re earch I stitute
U iversity of Michigan Medical S hool
An Arbor
M 48109 USA

ways as a chelate agent or as an SH group donor In the first case we have to assume that copper and other metals are necessary to taste sense: in the second that thiol groups interfere with taste acuity

The copper serum concentration in patients and animals treated with some drugs rich in thiols and exhibiting ageusia is normal yet copper administration restores the taste acuity Although both drugs have an identical chemical structure it is difficult to think in terms of a chelating mechanism in Homo-cysteine because it has been supposed that this drug does not deplete copper (Henkin et al 1969) Hence we wonder how it is that copper and other metals can restore taste sensitivity Perhaps they bind the excessive SH concentration for another mechanism

The preneural phase of taste could hypothetically be influenced by metals and or thiol concentrations that would regulate the effectivity of the contact between the tastants and the receptors Beidler (1954 1970) assumed that the gustatory stimuli interact with specific sectors of the plasma membrane of the receptor cell he characterized these sectors as receptor sites According to Vinnikov (1974) the most recent biochemical investigations have confirmed that the interaction of a taste stimulus with receptor sites is based on physical processes of the absorption type and not on enzymic processes this is also Beidler s point of view

The sensation of taste can be regarded as sensitivity to chemical substances in solution (Jenkins 1978) that have to make contact with the receptor site The hypothesized interaction between metals and thiols must be one of making that contact easier or more difficult.

In any case this first regulation would act upon the four taste qualities, as has been confirmed by clinical and experimental experiences affecting the acuity but not the quality

Murray & Murray (1970) consider the taste pit in which a little known dense substance exists to be the region of primary reaction in taste appreciation. The dense substance is considered by Vinnikov (1974) to be an analogue of ion-exchange resins which are capable of refining gustatory substances in such a way that their gustatory stimulating chemical source can interact with the receptor surface i e with the plasma membrane of the microvilli present in the sensory cells Consequently this substance must play a protective role simultaneously

Some of us (Ciges et al 1978) studied the dynamics of pit region and observing that the taste buds desquamation process regulate in some way the size of the pores and the amount of dense substance which covers the ends of all but the most extended sensory hairs

We attach great importance to this substance in the primary events of the taste process because it covers the receptor sites and we assume that the action of metals and thiols must be effected upon the dense substance It might be an equivalent to the mucus covering the olfactory epithelium both substances being chemical elements corresponding to the nature of the stimulus On the other hand physical senses such as hearing or labyrinth have physical devices such as tectorial membrane cupuli and otoconium which play an important functional role

In order to contribute to a better knowledge of the different biochemical processes—and particularly the interactions between metals and thiols—that probably take place during the first events of taste we have carried out a double investigation in two different fields.

First. Determination of copper concentration in ageusic animals by Acetyl-homocysteine and D-Penicillamine at different levels not only in serum as is usual but also in sensory tongue mucosa saliva, salivary glands liver heart and muscle Knowing that the liver plays a central role in the maintenance of copper homeostasis and its storage along with muscles we thought that it would be worthwhile investigating these organs

In tissues and body fluids there are true copper metalloproteins which are not in the same class (Sass-Kortsak & Bearn 1978) as

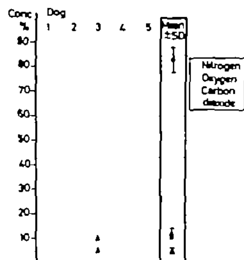


Fig 1 Composition of middle ear gas of air ventilated dogs (individual values mean and standard deviation)

(Sadé et al 1976) Under those conditions we were able to obtain usually 40–60 μl of MEG The gas samples were examined on the day of collection

The MEG composition was determined by a Packard 836U Gaschromatograph with a thermal conductivity detector using a combination of Porapak and 5A Molecular Sieve column system as previously described (Ostfeld et al 1979)

RESULTS AND DISCUSSION

The determinations were carried out on 5 dogs The composition of the MEG as obtained in the individual dogs is shown in Fig 1

The following mean content (±S D) of gases in ME of air ventilated dogs was found N_2 83 2% ± 5 O_2 12 1% ± 2 2 and Co_2 4 7% ± 0 7 The ranges were from 80 to 86% for N_2 from 10 to 15% for O_2 and from 3 7 to 5 5% for CO_2

The present study of the composition of the MEG in air ventilated dogs has been undertaken as a preliminary step in our investigation on changes occurring under various conditions of general anesthesia The dog has been chosen as an experimental animal because its ME volume is similar to that of humans and because it enables the use of an anesthetic technique identical with that used in clinical surgical work Hopefully the observations obtained in dogs may be valid and useful also for understanding the physiological processes related to MEG in man

During the sampling of MEG special attention has been given to the prevention of inadvertent leakage of air as cautioned by Drettner (1975) This was achieved by covering the TM with a 4–5 mm seal of saline (Sadé et al 1976)

Matsumura (1955) analysed the MEG composition of cats and men by Krogh s gasometric microanalytic method in which the volumetric percentages of the gases were determined following absorption of CO_2 by KOH and O_2 by an alkalized solution of pyrogallol. His results failed to demonstrate the presence of CO_2 in the MEG though allowing for an experimental error the possible presence of less than 1% CO_2 could not be ruled out. In the MEG of man O_2 was present in the range of 14–17 volume per cent. Similar O_2 content was found in normal cats (12 8–16 2%)

Melvill Jones (1961) analysed the pressure changes in the ME of humans after simulated flights in a decompression chamber during breathing of known gas mixtures By extrapolating the data he obtained values of normal near-equilibrium at approximately the 8% level corresponding to a 55 mm partial pressure of oxygen introduced into the middle ear for steady state condition The partial pressure of CO_2 was estimated to be 40 mmHg According to Drettner (1975) those results correspond to 9 3% O_2 and 5.5% CO_2 However Melvill Jones (1961) emphasized that in view both of the indirect method of approach and the limited data available the above results cannot be considered a very reliable estimate

Riu et al (1966) measured the human MEG composition by methods similar to ours namely gas chromatography of transtympanic MEG samples and found 9.5% O_2 and 5 5% CO_2

Sadé & Weisman (1977*a*) measured the CO_2 content of normal human MEG obtained after



Fig 4 Base of the tongue of one of the ageusic animals by Acetyl-Homocysteine. Observe the papillae intensely stained by osmium tetroxide

(from 9.52 to 12.03) In the rest of the organs the deviations of the values are not significant.

In the animals treated with D-Penicillamine in which ageusia did not appear the results were:

serum	8 44 γ/ml
saliva	18 78 γ/g
liver	31 14 γ/g
muscle	14 87 γ/g
heart	11.27 γ/g
gland	18 16 γ/g
tongue	8 60 γ/g

The fall in copper concentration in the liver as in the case of Acetyl-homocysteine is important (from 56 80 to 31 14) (Fig 1) The values for dispersion and variance are higher as far the liver is concerned (Figs. 2 and 3)

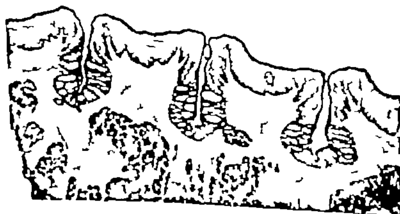


Fig 5 Semithin section of normal tongue of one of the ageusic animals, by Acetyl-homocysteine

above the equivalent of 40 to 45 mmHg pO_2 Melvill Jones (1961) found an early increase in the ME pressure during pure oxygen breathing in post flight experiments Our analysis of MEG after 30 minutes of post anesthetic ventilation of dogs with pure oxygen has shown a significant increase in the oxygen concentration in the ME from 12 to 19% (Ostfeld 1979*b*)

ZUSAMMENFASSUNG

Die Zusammensetzung der Gase im Mittelohr ist bei 5 normalen Hunden unter allgemeiner Anästhesie mittels Sodium Thiopentone untersucht wurden Die Gasproben wurden durch Trommelfell Punktion gewonnen und gaschromatographisch analysiert Folgende Gasmittelwerte ± Standardabweichung wurden erhalten. N 83.2±5 0% O_2 12.1±2.2% und CO_2 4 7±0 7%

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E. Ostfeld MD
ENT Department
Chaim Sheba Medical Center
Tel Hashomer
Israel

Acta Otolaryngol 89: 249–257 1980

HUMAN PITUITARY TUMOURS IN ORGAN CULTURE WITH CORRELATION TO CLINICAL DATA[1]

Matti Anniko and Jan Wersäll

From the Department of Oto-rhino-laryngology Karolinska sjukhuset and King Gustaf V Research Institute Karolinska Institutet Stockholm Sweden

Abstract Out of 56 operated pituitary tumours 31 were hypersecreting growth hormone (GH) 12 hypersecreting prolactin (PRL), 2 hypersecreting ACTH and 11 tumours were chromophobe without secretion of hormone. One pituitary revealed normal conditions at the surgical exploration later confirmed at the renewed endocrinological investigation. 36 pituitary tumours have been explanted to in vitro culture. 20 tumours causing acromegaly 9 prolactinomas, ACTH-producing and 5 chromophobe adenomas. The higher the GH concentration was in vitro the more active was the degree of acromegaly found clinically. GH hypersecreting tumours also secreted small amounts of PRL in vitro and the contrary concerning prolactinomas. The GH/PRL ratio was individual for each tumour GH or PRL hypersecreting. Tumour tissue revealed approximately the same characteristics in vitro irrespective of whether taken from different regions of the tumour. A good correlation occurred in the secretion of PRL in and in vitro the higher the serum level of PRL the higher concentration of PRL found in the culture medium. Mitoses did not occur in any of the histological sections of more than 400 separate specimens. The increase in PRL in cultured specimens is probably due to the absence of prolactin-inhibiting factor (PIF) during in vitro culture. On the other hand the decreasing GH levels may be due to the absence of GH-releasing factor. The results of the present study suggest an individual biology in each pituitary tumour.

Pituitary tumour disease presents a variety of signs and symptoms. Endocrinologically active tumours are nowadays generally diagnosed at an early stage especially prolactinomas in women, but large bone-destructive lesions of the sella turcica have still not disappeared in clinical work. Such entities are encountered especially in growth hormone (GH) producing tumours and constitute a serious problem in the surgical treatment.

Medical treatment of pituitary tumours has been introduced in some cases of tumours hypersecreting prolactin (PRL) (Besser et al 1977 Varga et al 1977 Thorner et al 1974 Thorner & Besser 1978 Bergh et al 1978) and in a few cases of tumours causing acromegaly (Liuzzi et al 1974 Thorner et al 1975 Besser et al 1976 1978 Cassar et al. 1977 Werner et al 1978) However the effect of this treatment with bromocriptine cannot be predicted and has to be tried in each case separately The response to bromocriptine shows great individual variations a fact that may later lead to surgery if the tumour grows during medication A special problem is constituted by bromocriptine induced pregnancy in women with a known PRL hypersecreting tumour The high oestrogen levels during pregnancy may induce a rapid tumour growth during this period of time (Griffith et al 1978 Besser & Moult, 1978)

At least a certain degree of an individual tumour biology may be suspected also in cases with the same type of hormone hypersecretion The present study analysed GH and PRL hypersecreting pituitary tumours in an organ culture system—independent of extra-pituitary regulatory mechanisms—in correlation with clinical observations of these tumours

MATERIAL AND METHODS

Material

The transmaxillary or the transseptal route has been used for the exploration of the pituitary gland. The operating microscope was used during all surgical interventions of the sella

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[1] Supported by grants from Karolinska Institutet.

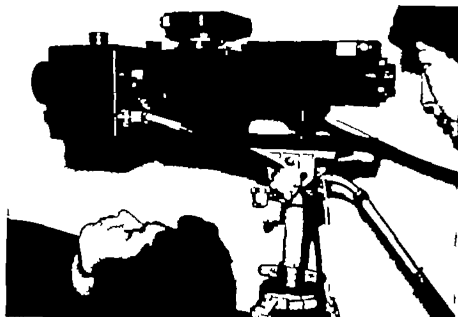


Fig 1 Binocular infrared pupillograph in use.

attached to the camera makes it possible to take two photographs per second at a shutter speed of 1/8 sec The depth of focus of this equipment is ±0 48 mm which is significantly smaller than the distance between the patient s eye and the film Thus its magnification rate in the case of a well focused photograph of the pupil can be considered to be stable Kodak HIE 420 was used as the film

MATERIALS AND METHODS

The patient was placed in a dark room where he rested for 10 minutes in a supine position Then five photographs of the pupils were taken using the motor drive The mecholyl test was conducted *ad modum* Loewenfeld & Oono (1966) One drop of 5% mecholyl was instilled into the conjunctival sac of each eye under illumination and another drop 2 min later at which time the light was extinguished Thirty minutes after the administration of the first drop five photographs were taken successively The film negatives thus taken were placed under a binocular microscope (×20), and the horizontal diameter of each eye was measured by the scale on the eyepiece (Fig 3) The real diameter of the pupil was obtained by multiplying the above figures by a correction value of the magnification rate (×0 13)

The pupil contraction rates were obtained in the following manner pupil diameter 30 min

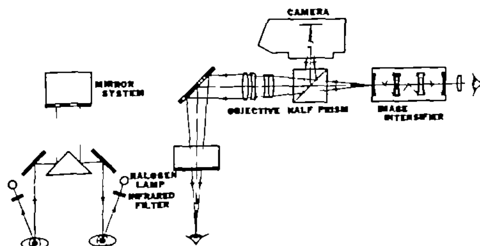


Fig 2 Block diagram of the binocular infrared pupillograph

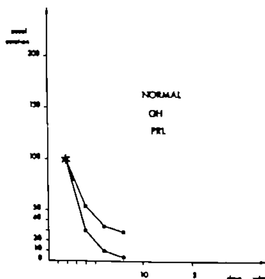


Fig. 1. Diagram illustrating the principles of GH and PRL synthesis/secretion from one normal pituitary gland in vitro. A difference is noted between GH and PRL secretion, the latter being more stable during organ culture but still decreasing with increasing time in vitro. The morphological processing revealed a large number of surviving cells in both groups of specimens.

Table III. *GH production in vivo and in vitro / clinical activity of the disease (acromegaly)*

Patient no	GH in pmol/l		Clinical activity of acromegaly
	In vivo[a]	In vitro[b]	
2	2 200	3 500	+
4	4 100	3 200	+
1	1 000	3 300	+
27	3 100	1 670	+
29	250	750	+
30	1 200	3 680	+
11	2 300	2 950	+ (+)
1	1 200	9 500	++
26	1 100	4 100	++
31	5 800	21 000	++
35	1 100	18 500	++
3	1 100	33 500	+++
5	2 600	35 000	+++
6	2 800	38 400	+++
14	1 900	900 000	+++
17	4 000	700 000	+++
20[c]	230	225 000	+++

[a] Mean levels (n=6–10) of fasting morning plasma GH.
[b] Mean levels of in vitro GH secretion (n=8–10) in pmol/l/48 h.
[c] Prior to surgery treated with bromocriptine for 3 months.

II. *GH hypersecreting tumours*

In spite of the fairly similar tumour-induced hormone levels in vivo, considerable differences appeared when explanting tissue from individual tumours to the organ culture system. The GH secretion into the culture medium followed one of two main patterns: maintained hormone synthesis/secretion at a fairly constant level for several weeks in culture or a gradually decreasing GH secretion with increasing time in vitro. During the first week in culture, specimens were occasionally found with an increased GH synthesis/secretion but the mode of secretion thereafter followed one of the two main patterns outlined above.

It was not possible to predict the in vitro synthesis/secretion of GH with regard to preoperative GH levels. However, the GH concentration in vitro was well correlated with the clinical activity of the disease. With regard to the clinical activity of acromegaly, the patients were placed in three different groups designated +, ++ and +++ respectively, the latter indicating the most active degree of acromegalic disease. Independently of this classification the secretion of GH into the culture medium could also be grouped into three different classes. It was found that the more active the clinical disease was in the patient, the higher the GH concentration occurring in vitro (Table III).

All tumours causing acromegaly also secreted PRL in vitro in varying concentrations individual for each tumour but only as a minor part of the total hormone synthesis/secretion. The ratio of GH/PRL was the same in specimens (N=24–36) from the same tumour but changed with increasing time in vitro (Tables IV and V). The synthesis/secretion of PRL in these tumours increased during the first 1–2 weeks in culture in approximately half of the tumours (Fig. 2). A few tumours revealed on the contrary a reduced secretion of PRL, whereas the remaining tumours had a fairly constant secretion of PRL into the culture medium.

Table II *The appearance of pathological miosis in normal and Meniere s disease groups*
The percentages in parentheses show the appearance rates of pathological miosis in each group

Pupil contraction rate	Normal	Meniere s disease: Interval stage, Unaffected side	Interval stage, Affected side	Quasi-attack stage, Unaffected side	Quasi-attack stage, Affected side	Attack stage, Unaffected side	Attack stage, Affected side
<10%	31	33	41	9	8	4	3
≥10%	1 (3 1%)	8 (19.5%)	15 (26 8%)	2 (18.[illegible]%)	8 (50%)	2 (33.3%)	5 (62.5%)
Totals	32	41	56	11	16	6	8

developed the condition while suffering from chronic otitis media and 3 after surgery of the middle ear For the vestibular neuritis group 4 patients who could be tested within 2 weeks after the onset were chosen The patients were also classified into attack interval and quasi-attack stages depending on the severity of the vertigo at the time of the testing

RESULTS

The pupil contraction rates of normal subjects and Meniere s disease patients are shown in Table I Although the rates among normal subjects were less than 14 9% those of the patients were often higher particularly on the affected side Borgmann et al (1974) in a study on the effects of 2 5% mecholyl on patients with pupillotonia stated that the pupil contraction rates of the unaffected eyes remained within 10% Therefore a pupil contraction rate in excess of 10% was considered pathological and the rate of appearance of pathological miosis was obtained



Fig 4 The pupils before and after mecholyl application in a patient with right-sided labyrinthitis

As shown in Table II the rate was 1/32 (3 1%) among normal subjects whereas on the affected side of the Meniere s disease patients it was 5/8 (62 5%) at the attack stage, 8/16 (50%) at the quasi-attack stage and 15/56 (26 8%) at the interval stage In the unaffected side among the patient group however these values declined to 2/6 (33 3%) 2/11 (18 [illegible]%) and 8/41 (19 5%) respectively The rates on the affected side of Meniere s disease patients at each stage differed from that of normal subjects ($P<0\ 01$) On the other hand no difference was found between normal subjects and the unaffected side of Meniere s disease patients ($P>0\ 05$)

Of the 5 patients with labyrinthitis 2 had developed the disorder while suffering from chronic otitis media. These 2 patients were tested 10 days and 1½ months after their respective onsets The remaining 3 had developed the condition secondary to stapedectomy for otosclerosis and radical mastoidectomy for otitis media. The mecholyl tests showed that no case had pathological miosis on the affected side alone In one case both eyes showed positive results (12 9% on the affected side and 12 2% on the normal side) and in another case the eye on the normal side showed positive results Fig 4 is a photograph of the pupils before and after mecholyl application in the case which had the severest vertigo among this group at the time of the testing The pupil contraction rate was 2 4% for the right eye and 7 2% for the left thus not pathological

tumours having a relatively stable GH and PRL secretion during at least one month in vitro Although the preliminary experiments by Landolt et al (1979) indicate that hypothalamic factors are needed for long-term survival and growth of explanted human pituitary tumours this is likely to be the case only when extending the culture period over several months

In prolactinomas there was a good correlation between the preoperative hormone level and the PRL synthesis/secretion into the culture medium in spite of the increasing synthesis/secretion of PRL with increasing time in vitro This indicates a causality with regard to the biological activity of the tumour Concerning GH hypersecreting tumours a causality is likely to occur between clinical activity of acromegalic disease and the in vitro level of GH

Each pituitary tumour revealed individual variations with regard to parameters investigated in vitro The biological behaviour of each tumour has therefore to be taken into consideration in the therapeutic management of the disease (acromegaly or hyperprolactemia) Recently Anniko et al (1979 to be published) demonstrated that the content of DNA in acromegaly varied according to the general aggressiveness of the tumour i.e clinical signs of activity duration of the disease bone destruction of the sella turcica etc Highly active tumours showed an aneuploid content of DNA whereas clinically more benign tumours were diploid It should be noted that cell mitosis is extremely uncommon as demonstrated in the present study Although the DNA content varies among individual pituitary tumours the histological findings reveal a benign type of cell morphology

There is little information on the effects of bromocriptine on the cellular or subcellular level of human pituitary tumours. Lloyd et al. (1975) von Werder et al (1978) and others have claimed that bromocriptine has an antiproliferative effect—at least with respect to PRL hypersecreting tumours. Acromegalic patients show a varying response to the drug in both acute trials and in long term treatment (Cassar et al 1977)

Halves (1978) and others have reported progress of tumour growth during bromocriptine therapy concerning both GH and PRL hypersecreting tumours Only a small number of cases have been published showing both a roentgenological regression of the pituitary tumour and a normalization of PRL levels following bromocriptine treatment (Vaidya et al 1977 Mühlenstedt et al 1978) Recently Wass et al (1979) published a series of 15 patients with PRL and 13 patients with GH hypersecreting tumours treated with bromocriptine only (prolactinomas 5–30 mg/day of bromocriptine for 8–78 months acromegalic patients 10–60 mg/day of bromocriptine for 6–52 months) In the patient group having a PRL hypersecreting tumour 3/15 showed both roentgenological evidence of tumour shrinkage and a normalization of the serum hormone level The patients with acromegaly had corresponding results in 2/13 It should be noted however that the acromegalic patients who showed roentgenological evidence of tumour regression were also hyperprolactinemic

An early tissue culture study on the inhibitory effect of bromocriptine on PRL secretion was reported by Pasteels et al (1971) (normal human pituitary gland) Subsequently Mashiter et al (1977) and Adams et al. (1979) have confirmed these findings also as concerns pituitary tumours Because of their technique (cell suspension) however it has been difficult to visualize the morphology of human tumour cells exposed to bromocriptine in vitro In experimental animals bromocriptine-induced changes in fine structure were shown as long ago as 1972 by Ectors et al

Tramu et al (1979) reported that degenerative morphological changes had occurred in three operated cases of PRL hypersecreting tumours where PRL levels had returned to normal 13–17 months after commencing bromocriptine treatment A correlation of

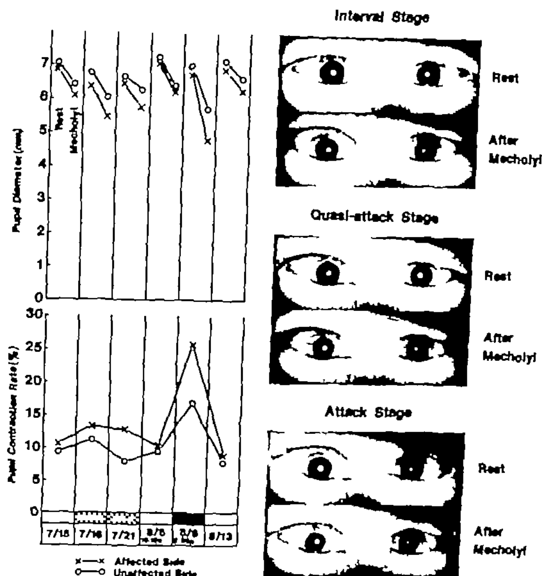


Fig 6 Case 2 Left-sided Meniere s disease showing left miosis after mecholyl application at the attack stage

Case 2
I S a 53-year-old male with Meniere s disease on the left side was first seen on July 9 1976 because of his nine year history of episodic rotary vertigo accompanied by left sided hearing loss and tinnitus In addition he experienced occasional attacks of a lateral swaying sensation of the body Around 3 a m on July 16 1976 he started to have such an attack The mecholyl test was conducted 9 hours later (quasi-attack stage) Again around 6 a m on July 21 the patient felt a similar dizziness and the mecholyl test was conducted 70 min later During these quasi-attack stages spontaneous nystagmus toward the affected side was observed Another mecholyl test was conducted at 10 10 a.m on August 6 du the interval stage At this time there was creased tinnitus on the left side but the pat did not have vertigo From 2 p m howe he began to have a severe vertiginous atta during which he felt as if his surroundi were flowing to the right The mecholyl t was started immediately Spontaneous nyst mus toward the unaffected side was obser at this time (attack stage) The mecholyl te conducted at the interval and quasi-atta stages showed very little difference in t pupil contraction rates of the left and rig eyes The test at the attack stage howeve showed strong miosis particularly on the le side (Fig 6)

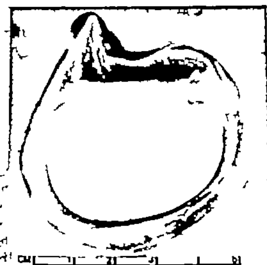


Fig. 1 Transverse section of the 2nd tracheal ring of male giraffe aged 15 years (VICTOR). Widest diameter 51 mm, shortest measurement 35 mm with tracheal length of 2.3 mm. Note the unequal lengths of the sides of the cartilaginous ring.

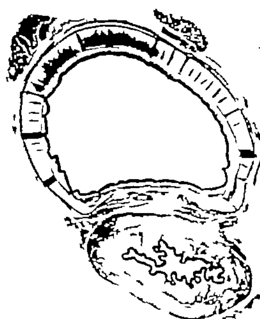


Fig. 2 Histological section of the tracheal ring shown in Fig. 1 showing the involuntary muscle lying posteriorly between the arms of the cartilaginous ring. This serves to diminish the tracheal lumen on expiration.

An assumption that these animals and others such as Deer Antelope Camels and Oxen are silent because they live in open country and can see each other ignores the need for purposeful vocal communication for survival Cries for help occur even in otherwise silent animals such as the Hare and Rhinoceros who squeal when dying or in fear

Warning of danger is of value for species survival and the sexual uses of sound are well known in the animal world including man The use of panic as an important factor in spreading alarm by the giraffe—thereby scattering the herd and diminishing the safety of animal grouping, suggests that useful sound is impossible

My continuing long term study of the detailed structure of the mammalian larynx (which now includes over 150 separate species) has provided an opportunity for examining the larynx and trachea of three specimens of giraffe Attention has been paid to the problem of the large volume of tracheal dead space conduction delay in recurrent laryngeal nerves which may be 2.5 m long and laryngeal structure

Respiration in the Giraffe

There is an important difference between respiration in ruminants and non-ruminating mammals Rumen gas contains not only nitrogen but varying amounts of methane oxygen and carbon dioxide Rate of gas production is variable possibly reaching 0 7 litres per minute but of considerable physiological importance is the amount of carbon dioxide produced for some of this is inhaled and absorbed into the lungs (Patterson et al 1965) Since mammalian respiration is designed to supply oxygen and remove carbon dioxide inhalation of ruminant carbon dioxide will affect breathing patterns Respiratory rates in giraffes in captivity have been noted by the author to vary from only 10 times a minute to over 45 times per minute when anxious Long necks contain long tracheas and this means a large respiratory dead-space Attempts have

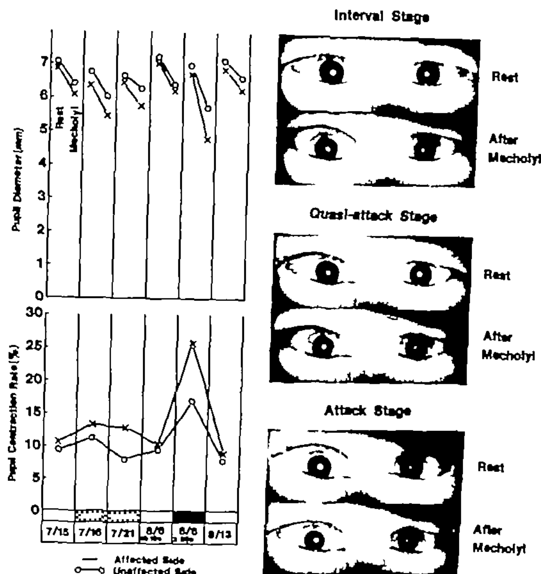


Fig 6 Case 2. Left sided Meniere s disease showing left miosis after mecholyl application at the attack stage

Case 2

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ured in this manner are rarely circular in out line and diameter was calculated as the diameter of the circle having a similar area to the fibre Both recurrent nerves in the large 15-year-old male have been studied sections being taken from the nerve as it passed beneath the inferior constrictor muscle Although the nerve was approximately twice the size of that found in humans much of this was found to be composed of connective tissue Total number of myelinated fibres was approximately 400 with over 40% having a diameter greater than 16 μm. As yet the pararecurrent nerves have not been studied and it may be necessary to examine the superior laryngeal nerve as well to obtain a composite picture of the laryngeal motor nerve supply Certainly this particular pair of recurrent laryngeal nerves appear to contain many less myelinated fibres than expected although the proportion of large fast conducting nerves was greater than in the human (Fig. 3)

Too great a significance must not be drawn from these findings for differences in the contractile characteristics of intrinsic laryngeal muscles may represent differences in the functional properties of their muscle fibres rather than variations in the diameter of their related motor nerve fibres. However the recurrent nerves of this particular giraffe did appear to contain a small number of fast conducting large myelinated nerve fibres

The Giraffe larynx

Although a detailed account of the histological appearance of this structure will be given in a further publication Fig. 4 illustrates a macroscopical view of the left half of an adult male animal It bears some resemblance to that of the Horse possessing many of the features of a herbivore capable of swallowing semi-liquid food whilst continuing to breathe The epiglottis lies above the soft palate and the large arytenoids surmounted by cartilages of Santorini, protect the laryngeal inlet by means of the attached thyro-arytenoid folds. There appears to be additional support to these folds from cartilages of Wrisberg. Attachment of the upper end of the anterior wall of the oesophagus to the cartilages of Santorini help to open the former during deglutition (Figs 4 and 6) The flat thyro-arytenoid fold is approximately 25 mm long and the slit above is shallow and probably does not represent a true division of the thyro-arythenoid muscle In the fixed specimen it is difficult to determine whether the arytenoids are fully abducted As in other ungulates they are hinged posteriorly producing a diamond-shaped opening when the tips are abducted In this specimen length of the arytenoids were 30 mm representing almost seven tenths the diameter of the glottis Negus believed that this represented otpimum conditions for glottic air flow and certainly the giraffe can equal the horse in a prolonged chase

An animal requires only a simple larynx to produce sounds The evolution of separate inferior thyro-arytenoid folds does not necessarily correlate with sound production for Bears with long, efficient folds use little voice neither does the Rhinoceros. However most animals make some definite purposeful sound under conditions of extreme stress and the Giraffe would appear an almost unique exception My investigations into the anatomical and physiological factors relating to respiration in this species suggests that this is not related to any unusual development in larynx or nerve supply It is possible that the basic underlying deficiency lies in the inability of this animal to produce an air flow of sufficient velocity to vibrate the thyro-arytenoid folds or arytenoids. This difficulty may be enhanced by the presence of a contractile trachea which in the Horse necessitates sound production on inspiration

ZUSAMMENFASSUNG

Trotz Besitzes einer gut entwickelten Larynx und einer sehr geselligen Natur kann die Giraffe nur ganz leise blöken oder lehren. Nach morphologischer und histologischer Untersuchung, sowie Messungen der Luftröhren und subglottisches Gebiets von drei frischen Larynges (*Giraffa camelopardalis*) konnten wir uns nun die Abwesenheit einer stärkeren vokalen Fähigkeit erklären.

the section of the nerve but takes a certain amount of time as reported in cases of denervated pupillary sphincter in the cat (Shen & Cannon 1936 Keil & Root 1941)

However the question has been raised as to whether the appearance of significant miosis is due merely to parasympathetic paralysis We have observed that the mecholyl test caused remarkable miosis in the affected eye of a patient with peripheral Horner's syndrome As regards the site of the underlying lesion it should be noted that in Adie s syndrome indicating characteristic supersensitivity to mecholyl the pathology was thought not to be situated in the periphery but centrally in the region of the hypothalamic vegetative centers (de Haas 1959) Therefore at present positive results in the mecholyl test should be interpreted as an indication of the existence of either peripheral or central autonomic dysfunction on the same side

There has also been some question as to whether the autonomic dysfunction observed in patients with Meniere s disease especially at the attack stage is directly related to the cause of the disease or not This is because changes in the peripheral labyrinthine function may induce autonomic dysfunction secondarily through the vestibular–autonomic reflex

Of the 4 patients in which significant miosis was observed on the affected side during the attack stage 2 were tested within two hours of the onset the remaining 2 during the attack or 5 minutes prior to it This may indicate the existence of autonomic dysfunction as the cause of the attack However the references on the time required for the development of supersensitivity have been obtained through animal experiments alone and observations on human subjects are expected in this connection in the future

In order to solve this question the test results were compared with those obtained with patients suffering from labyrinthitis and vestibular neuritis in whom a labyrinthine disorder was caused by the disease No one showed positive results limited to the affected side This eliminates the possibility [cut off] labyrinthine disorder alone can cause a[cut off] tive reaction of the affected side in the m[cut off] lyl test Therefore we can conclude th[cut off] autonomic dysfunction revealed by me[cut off] tests in patients with Meniere s disease [cut off] be present as a cause of the disease and [cut off] secondary to the occurrence of a labyri[cut off] disorder

ZUSAMMENFASSUNG

Um die Existenz einer vegetativen Dysfunktion [cut off] sache der Menièreschen Krankheit festzustellen, [cut off] wir Tests mittels Bindehautinstillation von Mecho[cut off] normalen sowie bei an Menièrescher Krankheit, an [cut off] rinthitis und an vestibulärer Neuritis leidenden Pa[cut off] durch. 1) Die Frequenz einer signifikanten Pupi[cut off] weiterung (großer als 10 %) war bei Normalen 3 1[cut off] signifikant höher bei den Menière Patienten an d[cut off] troffenen Körperseite im Anfalls- Quasianfalls[cut off] Intervallstadium Die Haufigkeit im kontralateralen [cut off] der Menière Patienten war in keinem der drei Stadi[cut off] ders als bei Normalen.) Keiner der Patienten mit [cut off] rinthitis oder vestibularer Neuritis zeigte eine signif[cut off] Miosis der involvierten Seite allein im Mecholyl Tes[cut off] bedeutet daß ein abnormaler vestibular autonom[cut off] Reflex an sich zu keinem positiven Mecholyl Test [cut off] Es kann daher geschlossen werden daß die Ursach[cut off] Menièreschen Krankheit mit der Existenz einer v[cut off] tiven Dysfunktion der lädierten Seite im Zusammen[cut off] steht.

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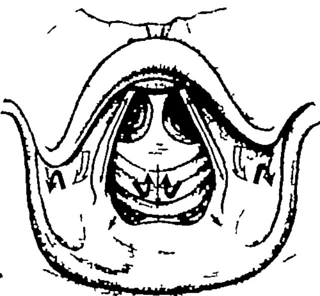


Fig. 2. Lymphatic drainage of the inner larynx, posterior aspect.

Electron microscopy

As during lymphangiography a morphological classification into lymph capillaries and collecting vessels is feasible. The *lymph capillaries* show a continuous thin endothelial layer with thickening in the nuclear area. The nucleous shows a double layered membrane with pores and a perinuclear cisterna. Within the cytoplasm there are mitochondria and many mostly longitudinally orientated fine filaments. There are multiple luminal and abluminal invaginations of the endothelial membrane and numerous smaller and larger vesicles within the cytoplasm. These vesicles seem to cross the cell similar to micropinocytosis. The endothelial cells are overlapping sometimes for a distance of a few microns with intercellular gaps measuring between 30 Å and 0.5 μm. When the junctions are pulled apart there is a rather free communication between the capillary lumen and the interstitial space.

The lymphatic *collecting vessels* show similar structural components except for two major characteristics. There is a collagenous adventitial layer with elastic respectively contractile elements and there are valves within the lumen. These valves are covered with an endothelium and the stroma is made out of loose connective tissue with collagenous and elastic fibres as well as fibroblasts.

DISCUSSION

Our experiments by means of lymphangiography and ultrastructural studies show that laryngeal lymphatics may be divided into lymph capillaries and collecting vessels. The function of this lymphatic system is to drain the interstitial space and by means of lymph angioscopy the drainage mechanism can be observed over longer periods. There are remarkable differences between in vivo and post mortem studies because in the latter case the dissemination of the dye depot in the interstitial space leads to a false compartmentation due to interstitial connective tissue bands.

Our lymphangiographic studies of the inner larynx demonstrate a sector-like centripetal lymph drainage via lymphatic collecting vessels into the regional lymph nodes. As multiple collaterals at the capillary and the collecting

Acta Otolaryngol 89 118–120 1980

MENIERE S DISEASE AND THE MASTOID PNEUMATIZATION

T Oku M Hasegawa and I Watanabe

From the Department of Otorhinolaryngology Tokyo Medical and Dental University Tokyo Japan

(Received April .., 1979)

Abstract The size of the mastoid penumatization was radiographically examined and planimetrically measured in the patients with Meniere s disease patients with other sensorineural hearing loss and clinically ear healthy persons Law projection was used for the radiographical examination No statistical differences were seen among the three groups. Meniere s disease and the factors which influence the mastoid pneumatization are discussed.

The aetiology of Meniere s disease is still un known However the histopathological finding is a distension of the endolymphatic com partments especially the cochlear duct and succule due to an increase in the volume of endolymph (hydrops) According to Schu knecht (1974) atrophy of the cochlear neurons of the apical turn vestibular fibrosis and epi thelial degeneration in the stria vascularis are other observation

Concerning the pathogenesis of endolymphatic hydrops the endolymphatic sac and vestibular aqueduct have been shown to play an important role (Yuen & Schuknecht 1972 Stahle & Wilbrand 1974 Arenberg et al 1977) The radiographical abnormality of the vestibular aqueduct which contains the endolym phatic duct and part of the sac was reported by Clemis & Valvassori in 1968 and they sug gested a correlation between an invisible aque duct and Meniere s disease Wilbrand et al (1978) suggested that periaqueductal pneu matization is sometimes poor or absent in the affected area of Meniere s disease

The aim of this study was to discover the total pneumatization of the temporal bones among the patients with Meniere s disease ... with other sensorineural hearing loss and clinically middle ear health persons and show whether or not statistical differences [are] seen among these three groups

MATERIALS AND METHODS

Twenty-seven patients with Meniere s disea[se] (16 males aged 23–55 11 females aged 2[?]–72) 27 patients with other sensorineural hea[r]ing loss (16 males aged 21–58 11 female[s] aged 28–74) and 25 clinically healthy perso[ns] (13 males aged 20–56 12 females age[d] 20–58) were radiographically examined On[e] of the 27 patients with Meniere s disease ... male had bilaterally affected ears Not in cluded with the patients with other sensori neural hearing loss were patients with sudde[n] deafness congenital syphylis perforated ea[r] drums anamnesis of acute or chronic oti[tis] media or anamnesis of tuberculosis

Law projection was used for the radiograph ical examination The size of the mastoid air cell system (cm^2) was measured by a plani meter The space of the middle ear was ex cluded from the measurement

RESULTS

The size of the mastoid air cell system in the patients with Meniere s disease patients with other sensorineural hearing loss and clinically ear healthy persons are shown in Table 1 No statistical differences were seem among Meniere s diseased persons and those having sensorineural hearing loss or those clinically

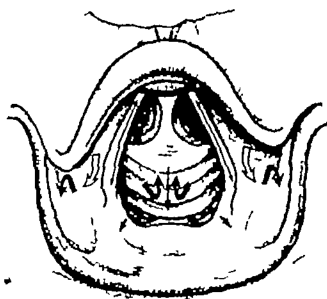


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cal factors for Meniere s disease or not by our radiographical examination of the temporal bone

RÉSUMÉ

La taille des cellules pneumatiques du système mastoïdien a été examinée radiographiquement et mesurée par planimétrie chez des malades atteints, de maladie de Meniere de perte d'audition neurosensorielle et sur des sujets dont les oreilles étaient saines. La projection de Law était utilisée pour un examen radiographique Aucune dif férence statistiquement significative n était trouvée parmis ces trois groupes La maladie de Meniere et les facteurs qui influeraient sur la pneumatisation mastoïdienne sont discutés.

ZUSAMMENFASSUNG

Die Größe der mastoiden Pneumatisation wurde radiographisch untersucht und planimetrisch gemessen in den Patienten mit Menierescher Krankheit den Patienten mit andere empfindungsnervlicher Gehörlosigkeit und bei Personen mit klinisch gesunden Ohren Lawprojektion" wurde für die radiographische Untersuchung angewandt Keine statistischen Verschiedenheiten waren unter diesen drei Gruppen Gemerkt. Die Menieresche Krankheit und die Faktoren die auf die mastoide Pneumatisation einwirken werden erörtert

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Makoto Hasegawa M.D
Departm nt of Otorhinolaryngology
Tokyo Medical and Dental University
1 5-45 Yushima Bunkyoku
Toky
Japa

segmentally directed by valves taking the way of least resistance In case of tumorous or infectious blockade or valvular insufficiency the segmental drainage is abolished and alternative routes lead to involvement of different laryngeal areas

ZUSAMMENFASSUNG

Lymphangiographische und elektronenmikroskopische Untersuchungen lassen am Kehlkopf eine Gliederung des Lymphgefäßsystems in Lymphkapillaren und Lymphsammelgefäße erkennen. Die letzteren unterscheiden sich von den Lymphkapillaren durch das Vorhandensein von Klappen sowie durch eine elastische Gefäßwand. Die Lymphangiographie zeigt, daß eine strenge Kompartmentierung einzelner Kehlkopfregionen nicht besteht. Ausnahme bilden die Lymphkapillaren des freien Randes der Stimmlippen Anastomosen der Lymphkapillaren und Lymphsammelgefäße erlauben mittellinien- und kehlkopfetagenüberschreitende Drainage Deren Kollateralabflüssen kommt bei Behinderung der physiologischen Lymphdrainage eine besondere Bedeutung zu

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Prof Dr Chl Beck
Doz. Dr W M
Univ Hals N sen-Ohrenklinik
Kilhenst
D-7800 Freiburg
We t Germany

DISCUSSION

Naumann to Beck We should be grateful to Mr Beck because through hi observations, he has made realize anew the possible risks of partial larynx resections. The existence of compartments and thus of anatomically proved boundaries for the expanding tumor in the soft parts of the larynx nevertheless play minor part in the indication of the *vertical* partial resections. However there is a different morphological basis for the principle of *horizontal* partial resections. I may recall that it was the *clinical* observations of the roentgenologist *Baclesse* which stimulated *J M Alonso* to develop his surgical concept of the horizontal partial resection. I may further bring to mind that embryologically speaking the laryn is developed by a cranial and a caudal part of what is later the windpipe coming togethe at the level of the laryngeal ventricle This leads in the area of fusion to kind of watershed and thus also to an effective barrier against cancerous growth It would be very bad for numerous patients if the result shown by Mr Beck were in consequence to lead to mistrust of partial resectioning of the lary The quality of life of patient who has had successful partial resection is incomparably better than that of patient who has had total laryngectomy The formation of a neoglottis (*Staffieri, Amatu*) unfortunately hardly changes anything here Partial resections of the larynx are often so helpful that their justification is beyond doubt as far as I am concerned. However their success depends greatly on very strict indication. This also is illustrated by M Beck esults.

Ehrenberger to Beck. Können Sie die Angaben anderer Autoren bestätigen daß die Membrana hyoepiglottica eine strikte horizontale Grenze zwischen der Lymphgefäßversorgung des Zungengrundes und des präepiglottischen Raumes darstellt?

Jakobi to Beck. Unter 1800 Hemilaryngektomien erreichten wir 80% Fünfjahresheilungen Nur wenige Rezidive zwangen zu Totalexstirpation Wie können die Differenzen zwischen den Untersuchungen des Lymphabflusses und den klinischen Erfahrungen erklärt werden?

Krajina to Beck. This paper is very interesting for people doing partial surgery on the larynx. I would like to make two remarks.

Doing the partial surgery of the larynx it is necessary to

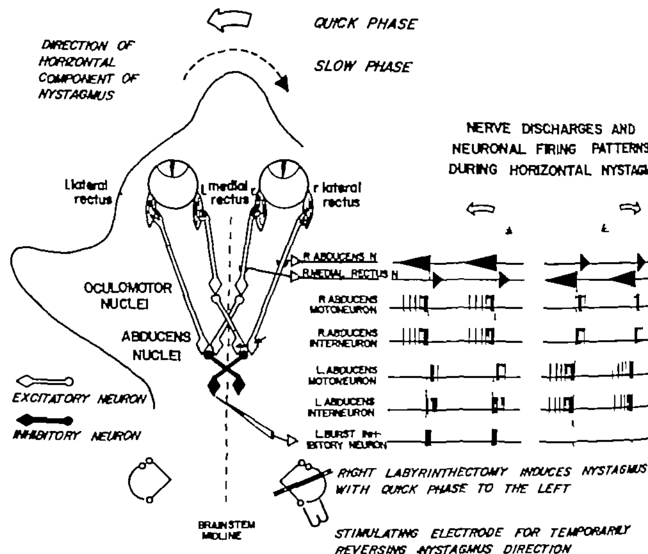


Fig 1 Schematic representation of some of the anatomical connections involved in horizontal eye movement control The neural firing patterns of these elements during horizontal vestibular nystagmus are shown on the right. The connections and firing patterns shown schematically here are derived from previous physiological studies of abducens motoneurons and interneurons (Baker & High stein 1975 Maeda et al 1972, Nakao & Sasaki, 1978 and burst inhibitory neurons (Hikosaka & Kawakami 1977 Hikosaka et al 1978*b*) The italicized letters describes the procedures in the present experiment.

activity of abducens motoneurons is brought about by the action of a small group of inhibitory neurons located around the caudal pole of the contralateral abducens nucleus (Hikosaka & Kawakami 1977) These neurons have been called burst inhibitory neurons (BINs) and we shall use that term BINs uniformly exhibit a short burst of high frequency firing (up to 800/sec) specifically at the end of the slow phase of horizontal nystagmus *only* when the slow phase is directed to the contralateral side They are silent or only fire sporadically during nystagmus in the opposite direction BINs are inhibitory and terminate on motoneurons in the contralateral abducens nucleus (Hikosaka et al 1978*b*) Consequently the burst of firing of these premotor neurons acts to terminate the slow phase activity of contralateral abducens motoneurons

Other cells in the pontine reticular formation have been shown to fire in relation to rapid eye movements (Luschei & Fuchs 1972 Keller 1974 Henn & Cohen 1976 Cohen & Henn 1972) However the exact role of these cells in the generation of rapid eye movements remains to be established BINs were spe-

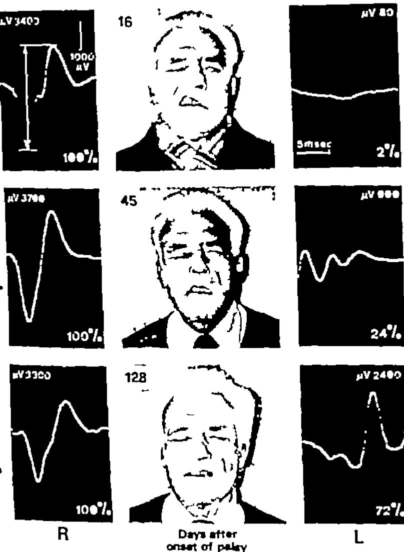


Fig. 2. Electroneuronogram of patient with facial palsy after parotidectomy

placed at the point where the facial nerve emerges from the stylomastoid foramen. Its two poles are set between the apex of the mastoid and the condyloid process of the mandible and in front of the tragus respectively.

The surface bipolar recording electrode is attached to the nasolabial sulcus and connected to the Mk III preamplifier. The test is carried out in a faradised booth though this precaution is not absolutely necessary.

A comparison is made between the responses of the unimpaired and injured sides to stimuli of the same intensity. A percentage estimate can thus be made of the number of fibres able to produce muscle contractions on the injured side.

The test is both quick and easy to perform. Patient tolerance is good. The final response consists of a roughly sinusoid wave whose positive–negative peak amplitude can be very accurately measured in mV to give the percentages required. Like all previous Authors in this field we evaluate the amplitude of the potential only. Latency and the area of the wave are of scant diagnostic and prognostic interest.

lution saturated with Fast Green FCF (1 2M ohm in resistance) were used for extracellular recording of unit spikes EEG was amplified with an AC (0 3 sec time constant) differential amplifier Extracellular unit spikes and compound action potentials of abducens and medial rectus nerves were recorded with single-ended AC amplifiers having 1 0 msec time constants These potentials were fed to an oscilloscope (Tektronix Type 5112) and when necessary recorded simultaneously on moving film magnetic tape and a strip chart recorder A comparator one-shot circuit was used to change the unit spikes into idealized spikes Firing rate was measured by low pass filtering of the idealized spikes or by a frequency to voltage converter (Curthoys 1978) A four-channel strip chart DC recorder (Gould Brush 2400) run usually at 25 mm/sec recorded BIN firing rate together with EEG abducens and medial rectus nerve discharges In some cases blood pressure was recorded instead of medial rectus nerve discharge

The anesthetic agent employed was 20% nitrous oxide in 80% oxygen Two respirators were used during the experiment The one already mentioned maintained the animal on normal room air Another respirator was connected through a Sweatman anesthetic machine for delivery of the nitrous oxide when necessary After recording in the pre anesthetic state for control the endotracheal tube was changed to the nitrous oxide delivery system which had been pre-charged with the above concentration of nitrous oxide to minimize dead air space The anesthetic was discontinued either when the burst firing of brain stem neurons became tonic or stopped or after 4–6 min of administration After recovery from anesthesia had been confirmed by EEG patterns the next neuron was studied with the same procedure Thus records of 2 to 4 neurons were usually obtained from each experiment The volume and rate of respirations were not changed before or during anesthesia. Normal physiological condition of the cat during the experiment was checked by ensuring that the concentration of CO_2 in the expired air remained at around 4% using a Beckman CO_2 analyzer

Recording sites of brain stem neurons were marked by electrophoretic ejection of Fast Green FCF (20 μA for 10 min) through the recording microelectrode (Thomas & Wilson, 1965) At the end of each experiment, the animal was sacrificed by intravenous injection of a lethal dose of pentobarbital and the brain stem was fixed in 10% of formalin solution. Frozen sections 50 micrometers thick were stained with Nissl-staining preparations.

RESULTS

Identification of BINs

Unit spikes were recorded extracellularly from the reticular formation in the vicinity of the abducens nucleus Neurons identified by the following characteristics were considered to be BINs (1) They were found in the dorsomedial part of the reticular formation at the level of or just caudal to the abducens nucleus and slightly lateral or inferior to the medial longitudinal fasciculus (MLF) (2) They exhibited bursts of firing exclusively at the quick phase of vestibular nystagmus directed to the ipsilateral side (3) They were silent or fired sporadically during nystagmus with quick phase in the opposite direction (see Fig. 1 Fig 2) This identification was based on the findings of Hikosaka et al (1977 1978*b*) These criteria excluded the possibility of recording other nystagmus-related neurons such as abducens motoneurons and internuclear neurons of the abducens nucleus and neurons in the prepositus hypoglossi nucleus and adjacent reticular structures which unlike BINs fire with a burst tonic pattern during both directions of nystagmus (Hikosaka et al 1978*a* Maeda et al 1972 Nakao & Sasaki 1978)

Sixty five neurons which satisfied these criteria were recorded Of these 30 neurons whose spike potentials were stable and well

of urgency. Rapid, efficient and dependable diagnostic techniques are essential therefore.

Electroneuronography is able to satisfy these requirements in a particularly effective manner. By comparison with other electrodiagnostic methods it has the prime advantage of offering early exact data that can be readily translated into quantitative values. If the nerve *deficiency* is more than 90% for example surgery may be undertaken less than 72 hours after the onset of traumatic paralysis since the simplicity and rapid execution of the test enables what amounts to a continuous watch to be kept on any progressive degeneration of the nerve.

Evaluation of course will depend on the aetiology. However once it is remembered that a facial defect does not become clinically evident until more than 40% of fibres cease to function, a test capable of detecting changes of only 5% is clearly a valuable guide in assessing both the further progress of a paralysis and the effects of treatment.

False negatives or positives are of course inevitable in all forms of diagnostic enquiry. Decisions with respect to treatment therefore should definitely be taken in the light of the evidence provided by several examinations, which should be repeated in further sessions wherever possible.

ZUSAMMENFASSUNG

Die Elektroneuronographie (ENoG) besteht in der Aufzeichnung eines durch einen supramaximalen elektrischen Reiz hervorgerufenen Summationspotentials der motorischen Einheiten. Die Degeneration (und offensichtlich die Unterbrechung) einer Nervenfaser bedeutet immer die Denervierung aller sich auf dieselben beziehenden Muskelfasern, so daß die Amplitudenabnahme des Summationspotentials der Anzahl der enervierten motorischen Einheiten verhältnisgleich sein wird. Dem Test liegt der Vergleich der Gegenwirkungen, je Reiz gleicher Stärke, zwischen gesunder und kranker Seite zugrunde und ermöglicht, die Anzahl der Nervfasern des verletzten Teils unzweifelhaft festzustellen, die gegenüber dem unbeschädigten Teil vermögen, das Zusammenziehen der davon abhängigen Muskelfasern hervorzurufen. Der Verfasser schildert die bei 60 persönlich beobachteten Fällen erzielten Ergebnisse und bespricht die unleugbaren von diesem [illegible] nostischen System gebotenen Vorteile bei der Beurteilung der Entwicklung einer Paralyse des Gesichtsnervs und bei der Festlegung genauer und vorzeitiger therapeutischer Angaben vor allem vom chirurgischen Gesichtspunkt aus.

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Prof. G. Rossi
Department of Audiology
Via Genova 3
10126 Torino, Italy

DISCUSSION

Stahle to Rossi. Have you any experience of measuring the area of the entire reaction besides the amplitude?

Jakobi to Rossi. Die guten Erfahrungen können wir nur bestätigen, meinen aber mit M. Portmann, daß weitere Parameter zur Beurteilung eines Verlaufes der Facialisparese notwendig sind. Der Fingerdruck, mit dem Rossi die Elektroden hielt, könnte eine Fehlerquelle sein. Wir bevorzugen deshalb für unsere sehr viel kleineren Elektroden den Heftpflasterkleber.

Pfaltz to Rossi. A single response in neuronography is not very reliable. For this reason averaged responses ought to be used for more reliable evaluation of the global facial response.

Ehrenberger to Rossi. Après nos expériences la neuronographie est une méthode éprouvée en cas des paralysies de Bell mais pas en cas des paralysies traumatiques même une perte totale de la fonction pendant la première semaine peut être suivie d'une restitution totale de la fonction nerveuse après plusieurs semaines.

Rossi (Reply).
Electroneurography is a very useful test in the diagnosis of facial palsy. Yet it is by no means the only electrical

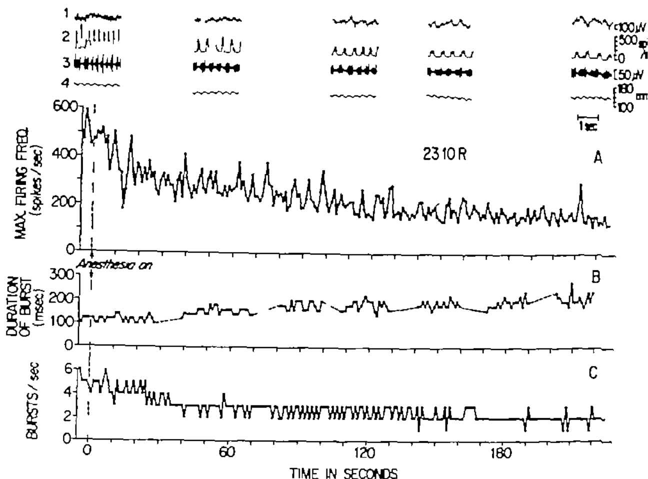


Fig 3 Changes of burst activity of a BIN in the early stage of anesthesia (A) Maximum firing frequency refers to the maximum firing rate of the bursts in each 1 sec interval (B) Duration of the burst in A (C) Number of bursts in each 1 sec Time scale for C applies to A and B Upper inset figures are traces of EEG (1) firing rate of the BIN (2) contralateral abducens nerve discharge (3) and arterial blood pressure (4) recorded simultaneously by the strip chart recorder Each set of the records was obtained at the time indicated in the abscissa of C.

lowed by a rapid decrease (A 3) At the end of the slow phase the BIN exhibited a high frequency burst of firing (sometimes more than 600 spikes/sec in the initial part of each burst) lasting 60–90 msec in duration (A 1) The onset of each burst approximately coincided with the time of abrupt cessation of abducens nerve discharge and preceded by 5–10 msec the sudden production of medial rectus nerve discharge When the direction of nystagmus was reversed by high frequency electrical stimulation of the vestibular nerve on the side of labyrinthectomy (B 2 and 3) the burst discharges of the BIN neuron were absent and individual spikes were rare (B 1)

In alert animals before administration of the anesthetic the maximum firing rate of BINs ranged from 220 to 880 spikes/sec and this peak rate occurred typically in the first 5 to 7 msec of each burst The duration of each burst was from 60 to 140 msec The number of bursts which corresponds to the number of nystagmic beats ranged from 2 to 4 5/sec

We wish to emphasize the uniform powerful inhibitory effect of BINs on contralateral abducens motoneurons BINs are typically silent at rest but during recovery from anesthesia, they fire tonically at a gradually increasing rate before returning to the pre anesthetic mode of short bursts separated by silence During this time of tonic BIN firing the activity of the entire contralateral abducens nerve was found to be governed by the activity of BIN cells Fig 2 D shows two cases where as the firing of just one BIN varies so the discharge of the entire contralateral abducens nerves varies in

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THE R THRESHOLD OF THE QUICK COMPONENT

A New Measure in Electronystagmography

M Pansini and I F Padovan
Zagreb Yugoslavia

Vestibular imbalance affects the eyes through the fasciculus longitudinalis medialis and oculomotor nuclei As regards vestibular excitation eyes could theoretically be turned around and around until balance is achieved However since physical and anatomical limitations do not allow such turning around the eyes could be held in the utmost lateral position in the position of conjugated deviation. Nevertheless the eyes are not held in this position of conjugated deviation since the compensatory central component brings the eyes back to the position of azimuth zero Logically this should depend on physical limitations and on the tension of the eye muscles which transmit information to the central structures via the proprioceptive afferent paths. The speed of the slow component and other elements also plays a certain role here It seems that we are faced with a great task of determining all these elements in order to define the point where the returning quick eye movement begins

There are many elements which send commands to revert eyes Sometimes the eye reverts after a high amplitude and sometimes after a low amplitude It is not the same in all individuals (Collard & Eber 1977) Many central mechanisms which are involved are not yet fully understood The number of the unknown elements is too large to allow any possibility of their definition and measurement (Fig. 1)

However the first step was carried out by determining the point where the slow component of nystagmus stops and the quick component begins as the point where the mechanism for reverting the eye starts to work. This point could be called the threshold of the quick component. This means that the point is identified but not explained Its identification makes it a possible subject for investigation

Identification and Measurement of R

The assumption that this point can be defined only by knowing all the elements which determine it, is quite discouraging. It *is* possible however to make a comparison between the many elements of nystagmus which are measurable even though their mechanisms are still not well known We may say that it is not absolutely necessary to know *all* the mechanisms in order to determine and measure this threshold On the contrary by making the threshold of the quick component measurable it serves for purposes of investigation and learning about the elements which determine it.

The next step in our exploration was made when we assumed that if there is a certain threshold of sensitivity for the appearance of the quick component, then it must be *constant* in the examined individual no matter whether nystagmus is weak or strong whether it is at the beginning in the middle or at the end of the provoked nystagmus This is called the law of preservation The Nobel Prize winner physicist Feynman says to a physicist, the law of preservation means the existence of a number which is definable at a certain moment and if we define it again after some time the number will not change—it will be of the same value no matter what changes occurred in the meantime

The threshold of the quick component was

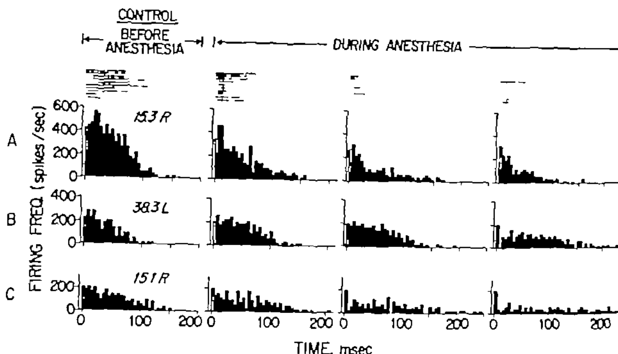


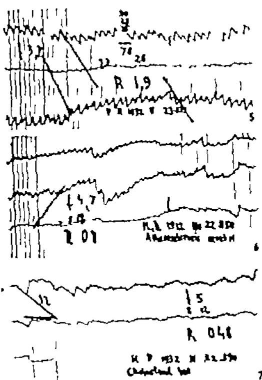
Fig 4 Histograms showing detailed changes in the characteristics of the burst for three BINs during anesthesia. (A) Each histogram was constructed by measurement in each 5 msec and averaging of dot plots of spike discharges of 10 successive bursts shown above it The first histogram during anesthesia was made from 10 successive bursts beginning 20 sec after the onset of anesth tervals of 20 sec elapsed between the later hist The time zero in each abscissa indicates the onse burst. The hatched bin shows the artificial con (see text) (B C) Constructed from other neurons

from 10 to 240 sec The mean time was 64 sec ± 54 (S D) $n = 22$ The time to the first detectable change in abducens nerve discharge (usually a decrease in amplitude or rounding of the end of the slow phase) ranged from 15 to 240 sec (mean 62±53 $n = 22$) Similarly the time to the first detectable change in medial rectus nerve discharge ranged from 11 to 150 sec (mean 62±49 $n = 13$) Changes in the BIN abducens and medial rectus nerve activity occurred at about the same time as the first detectable slowing of EEG (range of 15 to 240 sec mean 74±54 $n = 22$)

Fig 4 shows more detailed changes in the characteristics of the BIN burst for three neurons during anesthesia. Each firing rate histogram was constructed by measuring 10 successive bursts aligned at the first spike as shown by the dot plots for 15 3R at the top of the figure The average number of spikes in each 5 msec interval was multiplied by 20 to give the average firing rate in that interval The hatched vertical bar at the left of each histogram represents the artifactual addition firing rate resulting from using the first for alignment Before anesthesia the firin of all three neurons reached a maximum initial 10–30 msec of the bursts and gradually decreased One neuron retained pattern during anesthesia although the rate progressively decreased (A) In the two neurons the time to the maximum rate was prolonged with a progressive crease in firing rate and increase in the duration during anesthesia (B and C)

The later stages of anesthesia

After longer periods of nitrous oxide admi tration slow waves of 2–3/sec and/or a ness appeared in the EEG (Fig 6 A 1) In BINs their regularly repeated bursts v replaced by tonic firing with further an thesia, this tonic firing gradually decrease zero (Fig 6 A 2) Immediately before this to firing the maximum firing rate of bursts ran from 50 to 150 spikes/sec the burst durat



Figs 5–7 5 A case of atherosclerosis, with $R = 1.9$ 6 Another case of central atherosclerosis ($R = 0.8$) 7 A patient whose first measured R value was 0.5 It was subsequently found that his cholesterol value was 400

the illness progressed R increased to reach 64 in few months.

Angiopathies and R

In our investigations we noticed that the value of the threshold of the quick component decreases in central angiopathies It is less than 2 and in greater disturbances it is less than 1 (Figs 5–7)

Possibilities open to Investigation

Our investigations until now were limited only to the following two points (1) description and use of the new element in the electronystagmogram viz. the threshold of the quick component, and (2) explanation of the possibility of measuring this threshold and expressing its value numerically

Everything else presented in this communication is only the beginning of the work which in our opinion promises significant diagnostic possibilities

As an example for investigation we would like to mention a formula for fixational suppression

$$FS = \frac{NF\,(\text{non-fixation}) - F\,(\text{fixation})}{NF\,(\text{non-fixation})}$$

where usually the speed of the slow component is worked with but it is also possible to use the threshold of the quick component R We did it while examining a few healthy individuals and obtained the same values for the fixational suppression in s and R

Fixational suppression expressed in s was 85 1 and in R it was 85 7 It is interesting because in some pathological cases regarding fixational suppression there was no connection between values of the speed of slow component and the threshold of the quick component. It means that it is possible to discover disturbances in some mechanisms of fixational suppression and surely there *is more than one*

It is easy to construct a formula for calculating R in each individual nystagmic jerk We adjusted it to the speed of the ENG sheet of 15 mm/sec while for the speed of 10 mm/sec we should substitute the number 10 for 15

$$R = \frac{S}{\left(\frac{15}{N}\right)} \quad \text{or} \quad R = \frac{S}{\left(\frac{10}{N}\right)}$$

N = length of the nystagmic jerk, in mm

$\frac{15}{N}$ or $\frac{10}{N}$ = frequency of 1 jerk

This is a new datum which can help in the exploration of nystagmic irregularities where the change in threshold of the quick component is mostly involved

Various influences and effects on the threshold of the quick component can be explored effect of sedatives influence of the various levels of intelligence and motorics relationship with neurological diseases especially

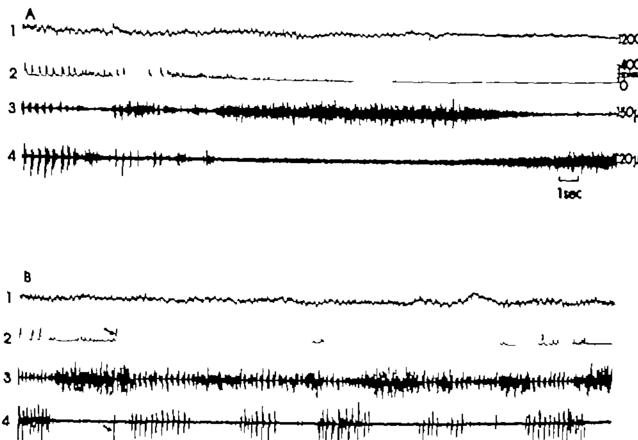


Fig 6 Change from burst to tonic firing of a BIN and its complete reduction of firing in the later stage of anesthesia. Simultaneous recording of EEG (1) BIN firing rate (2) and the activity of the contralateral abducens (3) and medial rectus nerves (4) during anesthesia. (B) To show the close relationship between BIN firing rate and medial rectus discharge The fluctuations in BIN firing are matched by fluctuations in the medial rectus discharge even to the extent that one BIN burst with a high firing rate (arrow) is accompanied by a medial rectus discharge. Same arrangement and calibrations as in A

Medial rectus discharge

One surprising result was a high correlation between the firing rate of single BINs and the strength of the medial rectus discharge at the quick return phase This correlation was usually observed in both unanesthetized and anesthetized animals Fig 6 B shows records from the animal in a period of the later stage of anesthesia in which rhythmic discharges of the abducens nerve tended to be replaced by tonic discharges (3) Whenever the BIN showed a high firing rate (arrow in 2) there was a strong discharge of the medial rectus nerve (arrow in 4) at the quick phase Conversely a strong discharge of the medial rectus was usually accompanied by a high firing rate in the BIN However the relationship was confined to the direction of nystagmus because BINs were nearly silent when the direction of nystagmus was reversed (see Fig 7 B)

DISCUSSION

The present study has shown that the character of vestibular nystagmus is quickly affected by nitrous oxide anesthesia and that one reason for this sensitivity is that the activity of a group of premotor neurons changes soon after anesthesia onset The burst activity of all the BINs tested was clearly changed after a short time (as soon as 10 sec) of anesthesia, most neurons responding with a progressive decrease in firing rate and number of bursts and with an increase of burst duration These changes were accompanied by a rounding or blunting of the end of the slow phase of the

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IMMUNODEFENCE OF THE INNER EAR?

Lymphocyte–Macrophage Interaction in the Endolymphatic Sac

Helge Rask Andersen and Jan Stahle

From the Department of Otolaryngology, University Hospital, Uppsala

Abstract. Owing to their proximity to areas exposed to infection, the sensory organs of the inner ear are probably dependent on an efficient antimicrobial defence. The longitudinal flow of endolymph to the endolymphatic sac may be of major importance in this context. Substances entering the ear can be automatically carried to the distal part of the endolymphatic duct where lymphoid cells are present and endolymphatic phagocytosis occurs. In the intermediate part of the epithelium of the sac morphological signs marking the incoming substances are present. A vigorous interaction between lymphocytes and macrophages, similar to that observed in antigen-activated lymphoid tissue, may be seen. The sac is here surrounded by a rich network of lymphatic capillaries and blood vessels.

The endolymphatic duct and sac are believed to absorb endolymph. Guild stated in 1927 that endolymph flows from the cochlear duct to the endolymphatic sac where it is absorbed and mediated to the numerous perisaccular blood vessels.

Phagocytosis by both the epithelial cells and free cells of the endolymphatic sac was demonstrated experimentally by Andersen (1948), Engström & Hjort (1950), Arnvig (1951), Lundquist (1965) and Rudert (1967).

The purpose of a phagocytic cell system inside the endolymphatic space is not known. Does the sac receive from the labyrinth effete cells or metabolic waste products too large and toxic for disposal via the cochlear duct? Or do the freely floating cells cleanse the sac of proteins normally concentrated therein and transport them via the epithelium into the surrounding blood vessels, as proposed by Ishii et al. (1966)?

Guild defined twenty-two species of cell element in the lumen of the endolymphatic sac. These included lymphocytes whose presence prompted Lim & Silver's (1974) proposal that the endolymphatic sac acts as an immunodefensive organ for the inner ear. Since toxins or foreign substances could reach the labyrinth through the windows of the middle ear cavity it could be crucial to inner-ear homeostasis that these substances are removed and eliminated by phagocytic cells in the presence of immuno cells.

In the present investigation lymphocytes and macrophages in the endolymphatic sac of guinea pigs were studied ultrastructurally. This was done in order to reveal a possible physical interaction which would indicate functional cooperation and could thus confirm the theory that the endolymphatic sac acts as an immunodefensive organ for the internal ear.

MATERIAL AND METHODS

Fifty pigmented guinea pigs ranging in weight from 200 to 800 g were used in this investigation. The animals displayed a normal Preyer reflex. They were anaesthetized with sodium pentobarbital (Nembutal) and perfused with a solution of 2.5% (v/v) purified glutaraldehyde and 1% (v/v) formaldehyde in a solution of 0.1 M Sørensen's phosphate buffer, pH 7.2–7.4. After decapitation the middle and internal ears were examined macroscopically for signs of infection. The operculum covering the endolymphatic sac was eliminated with a fine

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Supported by the Swedish Medical Research Council Project No. 03908

nerve discharge gradually diminished and surprisingly the medial rectus nerve discharge simultaneously increased (Fig 6A) Eventually the latter also diminished and in the deepest levels of anesthesia both nerves were silent (not shown in figure) This changing pattern could be explained as follows After acute labyrinthectomy say on the right side type I neuronal activity in the right vestibular nucleus is much reduced because of the right labyrinthectomy and strong commissural inhibition from the left vestibular nucleus In contrast the neuronal activity in the left vestibular nucleus is heightened because of release from commissural inhibition (Markham et al 1977 Shimazu & Precht 1966) In this condition and under moderate anesthesia the right abducens nerve discharges tonically This in turn is due primarily to the right abducens motoneurons receiving excitatory drive from the left vestibular nucleus (Baker et al 1969) At the same time the medial rectus is silent as a result of decreased excitatory influence from the right vestibular nucleus and its direct projection to toneurons (Baker & Highstein 1978)

Under deep anesthesia the right abducens nerve becomes silent and the medial rectus nerve fires tonically The first effect may be due to the anesthetic affecting the left intact vestibular nucleus and its direct projection to the abducens nucleus while the second effect results from lessened commissural inhibition acting on the right vestibular nucleus The reason the anesthetic acts on the vestibular nucleus on the intact side to a greater degree than on the side of the labyrinthectomy is presently unknown However the high firing rate of vestibular nucleus neurons on the intact side versus the abnormally low firing rate on the labyrinthectomized side may be a factor

While the changes in BIN activity during anesthesia apparently account for the changes in abducens nerve discharge it must be noted that we have no experimental data explaining the concomitant changes in medial rectus discharge at the quick return phase Through all stages of anesthesia there is a very close relationship between the firing rate of BINs a[illegible] activity in the medial rectus nerve (see Fi[illegible] 6B) This means that whenever there is a pa[illegible]ticularly abrupt termination of the slow phas[illegible] the ensuing quick return phase will be partic[illegible]larly strong

The medial rectus nerve discharge ori[illegible]nates from motoneurons within the medi[illegible] rectus division of the oculomotor nucleus. I[illegible] turn these motoneurons receive direct excit[illegible]tory input from the contralateral abducen[illegible] nucleus via the axons of abducens inter[illegible]nuclear neurons (see Fig 1 Baker & High[illegible]stein 1975) The cell bodies of these inter[illegible]nuclear neurons are on the same side as th[illegible] BINs studied here and are in close proximit[illegible] to them (Highstein & Baker 1978 Baker & Highstein 1975) However since BINs hav[illegible] been demonstrated to be inhibitory cells som[illegible] other neuron must be governing medial rectu[illegible] motoneuron activity in the quick phase o[illegible] nystagmus Sensitivity of these yet unidentified "governing" neurons possibly pauser neurons (see below) to anesthesia could explain the close relationship between BIN activity and medial rectus discharge during anesthesia.

The results reported here apply only for nitrous oxide anesthesia. In two other cats we used 2% halothane with similar results although the return from anesthesia was longer than with nitrous oxide It should be noted that the means by which drowsiness or sleep exert their effects on nystagmus may or may not be the same as for nitrous oxide

In this study we have shown that one reason rapid eye movements are so dependent on alertness is that one set of reticular pre-motor neurons controlling abducens motoneurons is quickly affected by anesthesia In turn one may ask why BINs are so sensitive It is known that they receive monosynaptic input from the contralateral superior colliculus and possibly also from cells in the gigantocellular tegmental field of the ipsilateral pontine reticular formation (Hikosaka & Kawakami 1977) They also receive polysynaptic input

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C H Markham M D
Reed N urological Research Ce ter
UCLA S hool of M dic ne
Lo Angele CA 90024
USA

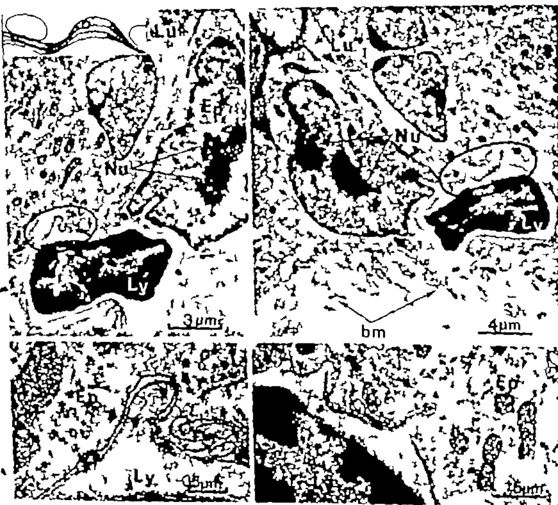


Fig. 6. Lymphocytes (*L*.) in the rugose epithelium of the *ES*. *Top*. The cells adhere to the epithelial cells (*Ep*) which display two nucleoli (*N*.). *Lu*. Endolymphatic lumen. ×2400 (*left*), ×1750 (*right*). *Bottom*. Pseudopodial processes project into the cytoplasm of the epithelial cells. There are small areas of subplasmalemmal density. ×9800 (*left*), ×5400 (*right*).

area of the intermediate portion of the sac were described with the help of light microscopy by Guild (1927) and ultrastructurally by Rask Andersen & Stahle (1979). They seem to be created when freely floating cell clusters fuse with the rugose epithelium (Fig. 3). They are rich in monocytes, macrophages and lymphocytes which probably originate from the perisaccular blood vessels. The epithelial crypts trap the cell cluster while the epithelium is invaded by mononuclear cells which later are presumably involved in the degradation of the luminal cells (Fig. 4). Lymphocyte–macrophage interaction is a common finding in these NVP (Fig. 5). The macrophages contain secondary lysosomes filled with digested material. Their large nuclei display numerous prominent nucleoli. There are broad areas of close contact between the macrophages and lymphocytes. The lymphoid cells sometimes penetrate deep into the cytoplasm of the macrophages.





Fig 1 (A) The neoplasm cords which infiltrated the larynx were surrounded by sclero-hyalin septa (G ×100) (B) The acidophilic tumour cells had a prominent nucleus and a granular cytoplasm (G ×250)

These nodes were not the metastases of an epidermoid carcinoma After a new examination a laryngeal chemodectoma with lymphatic and subcutaneous metastases was diagnosed Biological investigations were begun to study the functional features of this tumour the results are discussed below as well as those of the histological and ultrastructural analyses

In the following months no laryngeal recurrence was observed but the number of the subcutaneous nodes increased The radiographies of the lungs the bones the oeso-gastro-intestinal tract and intravenous urography did not reveal any anomaly The thyroid scintigraphy was normal The patient died at home on March 25 1975 and there was no autopsy

MATERIAL AND METHODS

Light microscopy Tissue blocks were fixed in bouin embedded in paraffin wax and 5 micron thick sections were cut These sections were examined after haematoxylin phloxin-safran periodic acid-Schiff and Masson s trichrome stain or after Fontana s and Grimelius s silver impregnations These paraffin sections were also used for the fluorescence tests for calcitonin and carcino-embryonic antigen research

Electron microscopy In addition small specimens (1 mm³) of the dorsal nodes were immersed in 1 77% phosphate-buffered glutaraldehyde and in osmium tetroxide The tissues were subsequently dehydrated b alcohol embedded in araldite CY 212. Ultrathin sections were contrasted with uranyl acetate and lead citrate The observation were made in an Hitachi 7 S or a Siemens Elmiskop 102 electron microscope.

RESULTS

Pathologic findings

Light microscopy The laryngeal biopsy consisted of a little necrotic mass in which

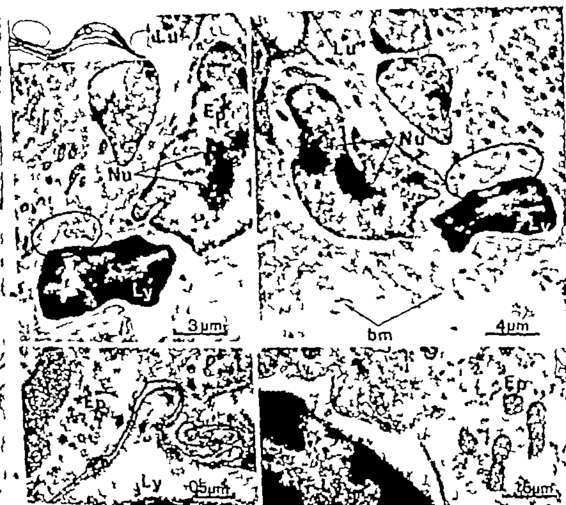


Fig. 6. Lymphocytes (*Ly*) in the rugose epithelium of the ES. *Top.* The cells adhere to the epithelial cells (*Ep*) which display two nucleoli (*N*). *Lu.* Endolymphatic lumen. × 2 400 (*left*), × 1 750 (*right*). *Bottom.* Pseudopodial processes project into the cytoplasm of the epithelial cells. There are small areas of subplasmalemmal density. × 9 800 (*left*), × 5 400 (*right*).

area of the intermediate portion of the sac were described with the help of light microscopy by Guild (1927) and ultrastructurally by Rask Andersen & Stahle (1979). They seem to be created when freely floating cell clusters fuse with the *rugose* epithelium (Fig. 3). They are rich in monocytes, macrophages and lymphocytes which probably originate from the perisaccular blood vessels. The epithelial crypts trap the cell cluster *while the epithelium* is invaded by mononuclear cells which later are presumably involved in the degradation of the luminal cells (Fig. 4). Lymphocyte–macrophage interaction is a common finding in these NVP (Fig. 5). The macrophages contain secondary lysosomes filled with digested material. Their large nuclei display numerous *prominent nucleoli.* There are broad areas of close contact between the macrophages and lymphocytes. The lymphoid cells sometimes penetrate deep into the cytoplasm of the macrophages.

Fig 3 Several lysosomal formations (*L*) near by secretory granules (*G*) mitochondria and filaments (G ×20 000)

encircled the clusters of tumour cells Here and there free granules were released be tween the stroma collagen fibers (Figs 2 3 4 and 5)

Biochemical investigations

The T C T -like activity was more than mg/ml Blood calcitonin reached 22 8? ng/ The fluorimetric dosage of the catecholam



Fig 4 A few desmosome (*D*) fixed the cells which were separated here and there by spaces filled up with microvilli (V) (G ×6 620).



Fig. 8. Plasma cells and lymphocytes in the distal region of the sac. This region of the perisaccular tissue is so rich in plasma cells in some animals that it may be called the 'plasma-cellular' region of the ES. *Top left.* Two mature plasma cells (*PC*) contain large amounts of rough endoplasmic reticulum and characteristic prominent perinuclear Golgi structures. Lymphoid cells appear as 'blasted' cells and are scattered nearby. ×1200. *Top right* and *bottom left.* The plasma cells (*PC*) have a close relationship to the reticular connective tissue cells (*Rc*). Numerous coated pits and vesicles suggest macromolecular transport in the reticular cell cytoplasm. ×5000, ×9400. *Bottom right.* Plasma cells (*PC*) surround large lymphocytes. Prominent mitochondria and a multivesicular body are seen. ×7500.

Perisaccular tissue

The intra-osseous portion of the endolymphatic sac is surrounded by an extensive network of post-capillary venules and veins. It is not unusual to observe small lymphocytes migrating through the thin endothelial walls into the loose tissue (Fig. 5A). Mast cells are widely distributed, many of them with pseudopods.

Fig 3 Several lysosomal formations (*L*) near by se cretory granules (*G*) mitochondria and filaments (G ×20 000)

encircled the clusters of tumour cells Here and there free granules were released between the stroma collagen fibers (Figs 2 3 4 and 5)

Biochemical investigations

The T C T like activity was more than mg/ml Blood calcitonin reached 22 82 ng/ The fluorimetric dosage of the catecholam



Fig 4 A few desmosome (*D*) fixed the cells which were separated here and there by spaces filled up with microvilli (*V*) (G ×6 620).

Acta Otolaryngol 89: 295–309 1980

OBLITERATION OF THE DUCTUS REUNIENS

Robert S Kimura, Harold F Schuknecht Carol Y Ota
and Diane D Jones

From the Department of Otolaryngology M   sachusett E   and Ear Infirmary and Harvard Medical School Boston M   sachusetts USA

Abstract The ductus reuniens was successfully obliterated in 57 guinea pig ears. Histopathological study showed that majority of these specimens demonstrated cochlear hydrops, saccular collapse and normal utricle. These results support the theory of longitudinal flow of endolymph from the cochlea toward the endolymphatic sac via the ductus reuniens and saccule. A major source of endolymph in the saccule appears to be the scala media. In another set of 11 animals in hich the ductus reuniens as first obstructed and two months later the endolymphatic duct was blocked endolymphatic hydrops as shown in the cochleae saccules, and utricles of all but one. The evidence suggests that cochlear hydrops was caused by obliteration of the ductus reuniens, and the saccular and utricular hydrops occurred subsequently as the result of blockage of the endolymphatic duct. Remnants of otolithic membrane which were attached to the distended saccular wall indicate that the membrane which had collapsed onto the macula after obliteration of the ductus reuniens is capable of subsequent distension. This experiment supports the concept of endolymph flow from the utricle and canals toward the endolymphatic sac A blocked ductus reuniens might also xplain the pathophysiological basis for the auditory form of Meniere disease.

There are two principal theories concerning endolymph secretion and absorption in the membranous labyrinth In the longitudinal flow theory endolymph of the cochlea is produced in the scala media and passes through the ductus reuniens saccule and endolymphatic duct to reach the endolymphatic sac where absorption takes place (Guild 1927 Lundquist 1965) Guild (1927) postulated that in the vestibular system there is a flow of endolymph from the utricle and ampullae through the utriculo-endolymphatic valve toward the endolymphatic sac. The radial flow theory contends that endolymph is both produced and absorbed locally within the scala media (Naftalin & Harrison, 1958 Lawrence & Mc Cabe 1959 Lawrence et al. 1961 Lawrence 1966) as well as in the utricle (Dohlman 1964 1965) The radial flow theory appeared to gain support from reports by Lindsay (1947) and Suh & Cody (1977) that no change in membrane position occurred after obliteration of the endolymphatic sac in the monkey and chinchilla respectively On the other hand the occurrence of consistent endolymphatic hydrops in other species of mammals after obliteration of the endolymphatic duct or sac (Kimura & Schuknecht 1965 Kimura 1967 1976 Schuknecht et al 1968 Beal 1968 and others) supports the longitudinal flow theory

Since the results of these experiments are clearly in conflict an experiment was designed to further test the validity of the radial or longitudinal flow theory in the guinea pig which is ideally suited for this type of experiment The experiment consisted of morphological studies on two groups of guinea pigs one with obstruction of the ductus reuniens alone and the other with obstruction of the ductus reuniens followed later by obstruction of the endolymphatic duct. The findings might also have relevance concerning individual dependence of the auditory and vestibular labyrinths on the absorptive function of the endolymphatic sac Furthermore the experiment might shed light on the pathophysiology of atypical forms of Meniere s disease It is known that endolymphatic hydrops is the principal pathological feature of this disease and that most affected patients will exhibit both auditory and vestibular symptoms Atypical cases do occur however where the symptoms and functional test

Fig 3 Several lysosomal formations (*L*) near by secretory granules (*G*) mitochondria and filaments (G ×20 000)

encircled the clusters of tumour cells Here and there free granules were released between the stroma collagen fibers (Figs 2 3 4 and 5)

Biochemical investigations

The T C T like activity was more than 10 mg/ml Blood calcitonin reached 22 82 ng/ml The fluorimetric dosage of the catecholamine



Fig 4 A few desmosomes (*D*) fixed the cells which were separated here and there by spaces filled up with microvilli (*V*) (G ×[illegible])

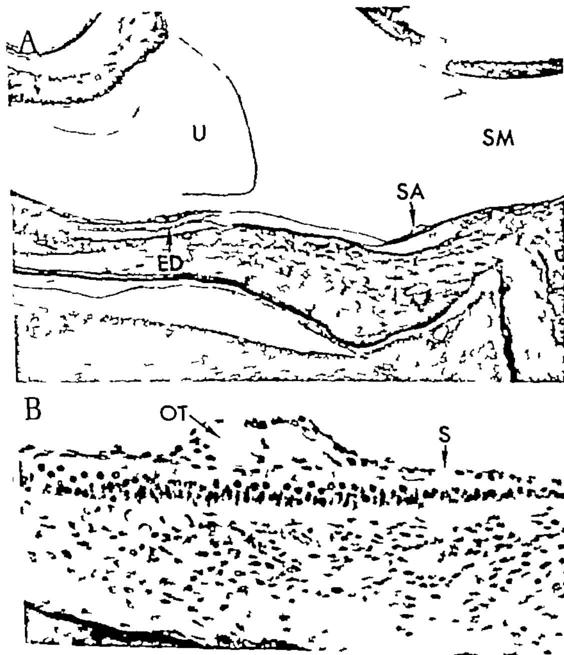


Fig. 3. The same specimen as shown in Figs. 1B and 2. (A) The saccular membrane is collapsed after ductus reuniens obliteration. The distended scala media (*SM*) is shown at the right. The endolymphatic duct (*ED*) is normal and patent. *SA*: Saccule; *U*: utricle. (B) A higher magnification of the macula sacculi in Fig. 3A showing the degenerated otolithic membrane (*OT*). The mass is surrounded by the saccular membrane (*S*).

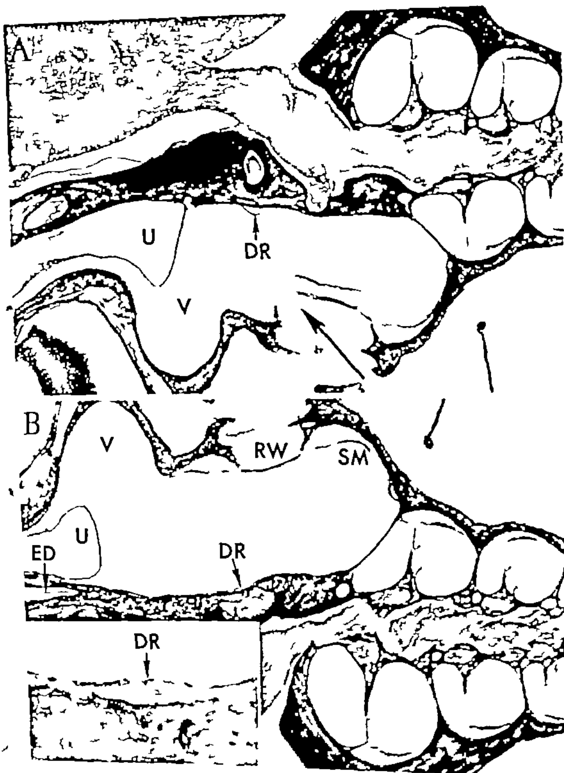
As controls of the experiment, the round windows were opened in six ears, the cochlear ducts were perforated through both Reissner's and basilar membranes in 13 ears and stapedes were mobilized or subluxated in seven ears. The saccular perforations performed in nine ears of an earlier experiment (Kimura et al. 1977) served as another experimental con-

Table I *The laryngeal chemodectomas*

No	Year	Author	Sex	Age	Evolution
1	1955	Andrews	M	77	No recurrence
2	1955	Blanchard & Saunders	M	38	No recurrence
3	1955	Zeitlhofer	F	56	No recurrence
4	1958	Schall	F	49	?
5	1960	Hartmann	F	40	
6	1962	De Barros	F	44	?
7	1965	Wockel et al	F	56	No recurrence
8	1965	Wockel et al.	M	56	Local recurrence
9	1965	Baxter	F	59	No recurrence
10	1965	Gignoux et al	F	21	No recurrence
11	1966	Otty	?		
12	1967	Martinson	M	14	No recurrence
13	1968	Azevedo-Gamas & Gloor	F	36	No recurrence
14	1970	Vetters & Toner	M	60	No recurrence after resection of the tumour and of a lymphatic metastase
15	1971	Benevant et al	F	30	No recurrence
16	1971	Ishida et al	M	19	?
17	1972	Adlington & Woodhouse	M	51	Dissemination with lymphatic and subcutaneous metastases
18	1972	Hooper	M	55	Dissemination with lymphatic and subcutaneous metastases
19	1972	Hooper	M	64	Dissemination with lymphatic and subcutaneous metastases
20	1972	Hooper	M	54	No recurrence
21	1972	Laudadio	F	[illegible]5	No recurrence
22	1972	Tobin & Harris	M	67	No recurrence
23	1972	Zachariah & Shah	M	61	Local recurrence and general dissemination
24	1975	Greenway	F	46	No recurrence
25	1976	Fraidooni	M	54	No recurrence after radiotherapy of the tumour and of a lymphatic metastase
26	1976	Markowska et al	F	42	No recurrence
27	1976	Piquet et al	F	51	No recurrence
28	1977	Lack et al	F	57	No recurrence
29	1978	Hohbach & Mootz	M	6[illegible]	No recurrence
30	1978	Perrin et al	F	41	No recurrence
31	1979	Justrabo et al	M	53	Dissemination with lymphatic and subcutaneous metastases

larger the inferior glomi found between the cricoid cartilage and the first tracheal ring were less affected All these bodies are composed of polygonal epithelial like cells surrounded by a vascular connective tissue (Fig 6)

This assertion is confirmed by the following facts The right aryepiglottic fold was destroyed in nine cases and the left one in three patients In seven other cases the left aryepiglottic fold the false cord the pyriform fossa the ventricle and the true cord were simultaneously colonized Three tumours infiltrated the right aryepiglottic fold the right false cord and the ventricle One was located to the right pyriform fossa and invaded the homolateral true cord two were subglottic and two supraglottic" The location in the four other cases was unknown For several months or years before the first examination only a hoarseness cervical pain and a dysphagia had been noticed in all the observations The indirect laryngoscopy showed a submucosal mass whose biopsy was often difficult haemorrhagic and unsuccessful Then a laryngo epiglottectomy or a tumourectomy were performed The neoplasm 1 5 to 2 cm in diameter could reach 6×4 cm (Andrews 1955 Tobin & Harris 1972) or was as small as rice-grain (De Barros 1962) It was limited by a capsule with a well developed venou


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ables ont déjà été rapportés. Les glomi laryngés supérieurs et plus accessoirement les glomi inférieurs sont le siège de ces tumeurs qui surviennent avec une égale fréquence dans les deux sexes Un enrouement est souvent le seul symptôme signalé pendant les mois voire les années précédant le premier examen de cette tumeur à croissance lente Néanmoins les chémodectomes laryngés se différencient des autres chémodectomes de la tête et du cou par un plus mauvais pronostic. Cinq des malades sont décédés dans un tableau de dissémination métastatique deux étaient porteurs de métastases ganglionnaires lymphatiques et un autre a présenté une récidive locale Les caractères ultrastructuraux et par fois l'activité fonctionnelle incontestable de ces chémodectomes sont ceux d'apudomes dont les cellules dériveraient d'éléments ayant migré de la portion céphalique des crêtes neurales jusqu au larynx

ZUSAMMENFASSUNG

Die Autoren berichten über einen Fall von Chemodectomus laryngum bei einem 53jährigen Mann Gestorben mit generaler Dissemination sowohl wie mit lymphatischen und unterepidermischen Metastasen Dieser funktionierende Neoplasmus produzierte große Mengen von Calcitonin bestimmt durch biochemische Untersuchungen und bei Immunofluoreszens. Vielleicht produzierte er auch adrenalinähnliche Substanzen Seine Zellen die den Hauptzellen des Glomus caroticum glichen waren mit neurosekretorischen Körnern und Lysosomen gefüllt Dreißig gleiche Fälle sind schon beschrieben worden. Diese Tumoren die in beiden Geschlechtern vorkommen sind in dem oberen Glomus laryngum und seltener in dem niederen anzutreffen Heiserkeit ist gewöhnlich während Monate sogar Jahre das einzige Symptom dieser langsamwachsenden Tumoren. Jedoch unterscheiden sich die laryngischen Chemodectome von den anderen Kopf oder Hals-Chemodectomen bei einer schlechteren Prognose. Fünf Patienten sind mit einem Bild von metastatischer Dissemination gestorben zwei trugen Lymphknotenmetastasen und ein Patient hatte lokales Rezidi Die Ultrastruktur sowie die zweifellose funktionale Aktivität dieser Chemodectome gleichen den Apudomen in welchen die Zellen wahrscheinlich von der cephalischen Neuralleiste abstammen

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In the series of 11 animals in which the endolymphatic duct was blocked two months after surgical obliteration of the ductus reuniens through the round window hydrops was observed in all cochleae saccules and in 10 utricles (Fig. 5) The magnitude of distension of these membranes was similar to that which follows blockage of the endolymphatic duct alone In 8 of 11 ears the distended walls of the saccules contained small remnants of the atrophic otolithic membranes structures similar to those observed on the collapsed saccular maculae in the series undergoing blockage of the ductus reunientes (Fig. 6) Other histopathological changes were neurosensory lesions at the basal ends or in both basal and apical turns similar to those seen in the ductus reuniens series Two specimens showed sensory cell atrophy in small areas of the saccular maculae. The sensory cells of the utricular maculae and cristae were normal except for for three posterior cristae which showed degeneration.

DISCUSSION

It might be argued that all these pathological alterations could be the result of a non-bacterial traumatic labyrinthitis and therefore are not related to obliteration of the ductus reuniens. Of course all invasive surgical procedures produce inflammatory and reparative responses One method of experimental control is to perform another invasive procedure which is similar but selectively different from the one under study When such controls were performed (opening of the round window perforation of the scala media, stapes mobilization) the resultant histopathological features were significantly different from those following obliteration of the ductus reuniens Even in our simple sacculotomy series (Kimura et al 1977) results were unlike those of the present ductus reuniens series Thus histopathologic observations in the majority of the specimens appear to be related to obliteration of the ductus reuniens Various other histopathological features in a small number of specimens could be attributed to labyrinthitis specimen preparation artifact tears in other parts of the membranous wall or simply non-healing of the fistulized membranes

The main histological characteristic after obliteration of the ductus reuniens was the combination of cochlear hydrops saccular collapse and normal utricle The combination with a much lower incidence was cochlear hydrops and normal saccule and utricle The cochlear hydrops may be explained if we presume that endolymph normally flows toward the ductus reuniens saccule and endolymphatic sac. If the ductus reuniens were blocked and endolymph were still being produced then Reissner's membrane would distend Supporting evidence from another experiment is the demonstration of cochlear hydrops after blockage of the endolymphatic duct, as stated earlier In a labyrinthotomy experiment in which the hook region of the cochlea was surgically destroyed the remaining parts of the cochlea developed hydrops (Hoshino & Paparella, 1970) Gussen (1978) reported that atrophy of the human macula sacculi resulted in accumulation of otoliths or otolithic membrane debris in the ductus reuniens and/or in the cochlear duct The tissue reaction at these areas caused development of hydrops in the basal turn and even throughout the cochlea.

The saccule in the present study collapsed possibly because it was deprived of its main source of endolymph from the scala media. There are no secretory cells (dark cells) in the saccule (Kimura, 1969) A close physiological relationship between the cochlea and saccule was also shown by the collapsed Reissner's

Fig 6 The saccular membranes of specimens in which the ductus reuniens was obliterated first followed by blockage of the endolymphatic duct two months later (A) The saccular membrane with the remnant of the otolithic membrane (*OT*) is partly lifted in the hydropic saccule (*SA*). *ST* Stapes footplate (B) The remnant of the otolithic membrane is attached to the distended saccular membrane. The same specimen as shown in Fig. 5C *EN* Endolymph side (C) Another specimen showing the lifted degenerated otolithic membrane attached to the distended saccular membrane *EN* Endolymph side

Acta Otolaryngol 89 144–151 1980

DYSPRAXIA OF SPEECH AND OF EYE MOTILITY

A I Dahlen S Fex N G Henriksson I Pyykkö and C Wennmo

From the Divisions of Otoneurology and of Phoniatrics Department of Otolaryngology University Hospital Lund Sweden

(Received January 8 1979)

Abstract Five patients with a disturbance of their pre programming of speech (dyspraxia of speech) were exposed to a comprehensive eye-motor test-battery The saccades were found hypometric and inaccurate with irregular intervening pauses The finding was interpreted as due to an extension of lesions from frontal cortical speech areas into visual motor cortex disturbing the normal preprogramming of voluntary saccades The results were interpreted as supporting the assumption that voluntary saccades are initiated in the frontal cortex An increase of vestibular nystagmus in these cases was related to a release of vestibular nystagmus due to the disappearance of cortical inhibition on brain stem activity

This paper will deal with disturbances in the same patients of two seemingly unrelated voluntary activities that of preprogramming of speech and that of preprogramming of voluntary fast eye movements the voluntary saccades Both types of activities demand the will of the patient as well as a large amount of coordination of muscle activity and both activities may be triggered in the frontal cortex in front of the pre-central gyrus

This specific disturbance of programming of speech may be due to a lesion in the frontal cortex resulting in a typical speech pattern (Darley et al 1975) In these cases although the persons are capable of finding the right word from their "bank of words they are unable to initiate a proper combination of muscle activity to produce the word Instead they will produce with a normal tone and articulation and normal strength faulty constructions of that word They are aware of their mistakes and will repeatedly try to correct themselves frequently without success

This disturbance of speech has been called apraxia (Liepman 1914 Darley et al 1975) Since this term would indicate a complete inability to control speech which rarely is the case we have suggested for this disturbance and have in this paper used the term dyspraxia.

In these patients a pronounced decrease of accuracy of fast eye movements was frequently found These patients seem to have lost their normal ability to preprogram voluntary eye movements together with their ability to preprogram speech This combination initiated the present study of various types of eye movements in patients in whom the dyspraxia of speech indicated a frontal cortical lesion

MATERIAL AND METHODS

Five patients with a speech disorder of the dyspractic type were examined and compared with five age matched normal subjects (Table I) The speech disorder was classified according to conventional phoniatric tests (Johns & Darley 1970) Great care was taken to exclude patients with impressive or expressive aphasias from the study However in all patients with dyspraxia a component of expressive aphasia could be detected This usually was of a minor degree when compared with the disorder caused by motor dyspraxia.

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Robert S. Kimura Ph.D
Department of Otolaryngology
Massachusetts E and Ear Infirmary
43 Charles Street
Boston Massachusetts 02114 USA

DISCUSSION

Wersall to Kimura I have always admired Dr Kimura xperimental surgical ability I want to congratulate you on the quality and consistency of your studies I find your present work very important for the understanding of endolymph circulation I have only one question. W know now that the dark cells are only found in the areas in the vestibular part of the labyrinth where we have endolymph production. Can you speculate on the function of the dark cells

Spoendlin to Kimura What is the size or rapidity of endolymph flow toward the endolymphatic sac? The second question is what is the surgical approach to the ductus reuniens through the estibule?

Sadé to Kimura Your excellent experiments leave little doubt that there is an endolymphatic flow from the cochlea to the endolymphatic duct and sac, and that blocking of this pathway results in distention (hydrops) proximal to the obstruction However as surgeon I must ask myself as to the functional meaning of the endolymphatic duct (or sac) obstruction Because I (like other ear surgeons) have seen and published cases where slow bone resorption associated with deep cholesteatoma has destroyed the entire labyrinth—at times not sparing the vestibule wall (sparing only the cochlea), while the hearing before as after the operation showed normal or near-normal bone conduction. In these ears the endolymphatic duct as obviously included in the destructive process. If such ears developed histological endolymphatic distention—the so-called "hydrops"—then this hydrops caused them little harm. This tallies ell with the observation that such endolymphatic dilatation as found in Meniere patients is also found in multitude of other ear diseases or indeed at times in ears with no history of deafness or vertigo.

It would therefore seem that narrowing or closing of the endolymphatic duct and sac might be less dangerous than is usually assumed—and that the histological picture of a dilated endolymphatic ystem is unspecific and possibly does not alw ys signify pathological process

The connotations of the above to the various treatments applied to patients suffering from vertigo are self-evident.

Thalmann to Kimura I am particularly impressed by the collapse of the saccule since this is further proof of the secretory role of the dark cells which according to your previous studies are lacking in the saccule Also the resting potential of the saccule ha been show to be merely remote manifestation of the endocochlear potential Your xperimental pathological results agree beautifully w th current electrophysiological concepts about the role of the saccule.

Kimura (Reply):

T Wersäll I am very grateful for your nice compliment. At present I cannot give you more information on the function of dark cells I am sure that future study will reveal more specific function of the dark cells.

T Spoendlin It is my impression that the size of the endolymph flow toward the endolymphatic sac is rather small and slow This opinion is based on my experience of opening the normal endolymphatic sac However it should be noted that hydrops develops in the cochlea and saccule within 24 hours after obliteration of the endolymphatic duct. The surgical approach to the ductus reuniens through the vestibule is to open the bony wall posterior to the round window This is accomplished by thinning the bone with burr and deep circular groove is made with a pointed instrument. The bony disc and fragments are lifted out with fine hook. A long curved needle is inserted to cut the ductus reuniens of which the location was predetermined by the previous anatomical study

T Sadé I do not have an answer to your clinical observations. The histopathological study related to the present investigation is case of hydrops limited to the cochlea ithout having the typical symptoms of Menie re disease as reported by Lindsay and von Scholthers (1958) Gussen reported last year that cochlear hydrops was noted in the cases of cochlear and saccular degeneration. She attributed hydrops to the degenerated otolithic membrane entering into the ductus reuniens and the cecum vestibulare where fibrosis and inflammatory reaction developed.

T Thalmann. Collapse of the saccule membrane is a real phenomenon. I am still convinced that there are no dark cells in the saccule The main source of endolymph is probably the scala media. Distribution of dark cells in the vestibular labyrinth is mapped in my previous publication. They are shown in all three ampullae and utricle. A part of endolymph in the saccule may come from dark cells of the utricle and ampullae

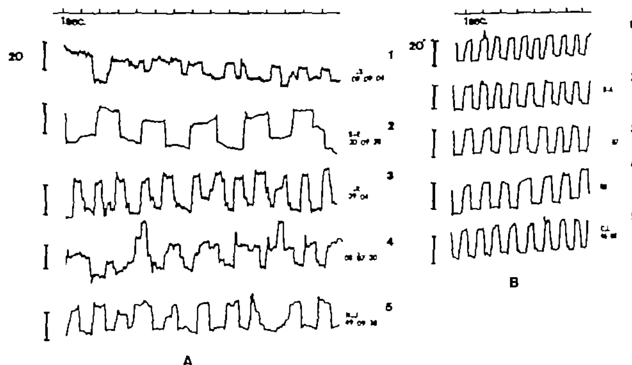


Fig 1 The command eye movements of 20° (A) in patients with dyspraxia (B) in healthy controls. The time scale is shown in the uppermost curve in pictures

patients 3 4 and 1 The same patients showed a disturbance of following eye movements When comparing the optekinetic patterns of normals and of the diseased there were no clear-cut differences However the amplitude of optokinetic nystagmus of the patients was slightly larger than that of the normals

4 *The eye movements in rotation tests*

Vestibular nystagmus induced by rotation in darkness was normal in all patients except no 4 in whom the amplitudes were very low The nystagmus was rhythmic especially so in patients 1 and 5 (Fig 4) The average amplitude was larger in the group of patients than in the group of normals

5 *Eye movements in caloric tests*

The nystagmus caused by caloric irrigation of the ears in the dark was remarkably regular and quite symmetrical in all patients (Fig 5) The amplitudes of the normals were definitely smaller than those of the patients A nystagmus of remarkably high amplitude was found with in patients 1 3 and 4 These patients showed a disturbance of the fol ing movements and a lower amplitud optokinetic nystagmus than the two ren ing patients

In an effort to evaluate the correlatio tween the different nystagmus responses the pursuit ability four characters ranked for comparison the smoothnes the following movements and the amplit of caloric optokinetic and postrotatory tagmus In this way the amplitude of cal and of optokinetic nystagmus could be ted against smooth tracking ability (Fig This plotting indicates that caloric an tudes decrease with increasing tracking ab while optokinetic nystagmus increases this ability The postrotatory nystag pattern was so similar in four cases tha ranking could be made

RESULTS

Patients with dyspraxia of speech prod saccades of normal velocity but with nounced dysmetria and with prolongatio pauses between saccades In spite of this

Unseren Befunden nach ist sie ein Teil der herdförmigen perilymphatischen Fibrosierung die beherrschendes morphologisches Substrat dieser Erkrankung ist

4 Die Veränderungen an der Stria vascularis möchten wir nicht wie Wolff und Mitarbeiter als Hauptursache des Hydrops anführen Sie könnten sicher beitragen durch vermehrte Lymphproduction was aber bis heute nicht beweisbar ist

Unsere morphologischen Befunde erlauben aber auch Rückschlüsse auf die Ätiologie der Erkrankung Da unser Fall allerdings kein akuter war können wir zu den sehr bemerkenswerten Befunden erhoben von Edström und Vahlne nicht Stellung nehmen. Wenn wir allerdings die Beobachtungen an den Gefäßen im Felsenbein als panarteriitis-nodosa ähnlich festlegen so wird die Störung im Immunsystem, über welche sie als Infektionsfolge berichten sehr wichtig. Auch unser Patient erlitt den ersten Schub des Hörverlustes im Rahmen einer Infektion Ob und wie eine Autoimmunerkrankung daraus entstanden ist können wir mangels Befunden an anderen Organen nicht festlegen Aber die Veränderungen an den Gefäßen rund um den Nervus facialis und an der Arteria labyrinthi sowie die Thrombosen in den Venen der Bogengangswand und der Begleitvene des Aquaeductus vestibuli können als pathohistologischer Beweis gelten. Wir möchten deshalb Oliner und Mitarbeiter sowie Fisher und Hellström unterstützen und auch Cogan und Dickersen, die annehmen daß das Cogan Syndrom eine Teilform der Panarteriitis nodosa ist, ähnlich der Wegener'schen Granulomatose in unserem Fachgebiet Somit wäre das Syndrom den Autoimmunerkrankungen zuzurechnen, wofür auch die Therapieerfolge mit hochdosiertem Cortison berichtet von Smith 1970 sprechen würden.

ZUSAMMENFASSUNG

Es wird über pathohistologische Befunde erhoben an einem menschlichen Felsenbein beim Cogan Syndrom berichtet. Die hervorstechenden Beobachtungen sind: 1 Fibrose im perilymphatischen Raum Scala vestibuli cysterna perilymphatica vestibuli rund um den oberen Bogengang und den Ductus reuniens. 2. Ossificationen in perilymphatisch-fibrosierten Arealen: Scala vestibuli der basalen Schneckenwindung, Vestibulum. 3 Zerstörung der Sinnesendstellen: Corti'sches Organ macula sacculi und utriculi 4 Endolymphatischer Hydrops mittlere, obere Schneckenwindung, Sacculus, Utriculus oberer Bogengang. 5 Perisacculäre Fibrose im Ductus endolymphaticus und Blockade der Bast'schen Klappe 6. Panarteriitis nodosa ähnliche Gefäßveränderungen an der Arteria labyrinthi den Venen um den Aquaeductus vestibuli und an allen Gefäßen um den Nervus facialis. Auf Grund dieser Veränderungen nehmen wir an daß es sich beim Cogan Syndrom um eine localisierte Erkrankung aus dem Formenkreis der Autoimmunerkrankungen handelt.

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Prof dr G Zechner
Hals Nasen- Ohrenheilk.
Wolkersbergenstr 1
A-1130 Wien
Austria

DISCUSSION

Keleman to Zechner Remarks of birth of Cogan syndrome at the Massachusetts Eye & Ear Infirmary until its knowledge grew as shown in the masterful presentation.

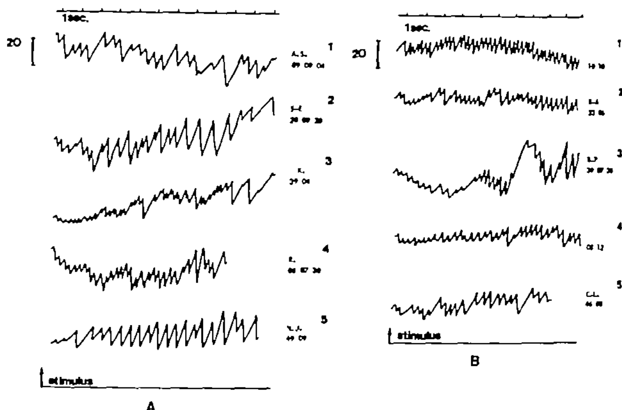


Fig 3 The optokinetic eye movements (A) in patients with dyspraxia, (B) in healthy controls

normal force The corresponding dimension in eye movement should be the velocity The velocities of the disturbed saccades were accordingly found normal in spite of the splitting up into many saccades

C *Tempo* A certain prolongation between consecutive letters syllables or words seems typical of the dyspractic speech The tempo is staccato with long intermissions during which the patient seems to search for the appropriate word or syllable In eye movements this pattern is reflected by prolongations of or variations in the periods between consecutive saccades

We may now claim at least that these three features of speech accuracy strength and tempo are reflected in corresponding features in patterns of eye movements

2 *Location of excitatory neurons for voluntary saccades*

Earlier concepts about the role of cortex initiating eye movements were largely based on clinical observations (Holmes 19 Cogan & Adams 1953) ablation stu (Latto & Cowey 1971 Pasik & Pasik, 19 and observations of eye movements indu by stimulation of the cortex (Ferrier 18 Penfield & Boldrey 1937 Pasik & Pa 1964) All these studies indicated that vol tary saccades are probably triggered by a 8 of the frontal cortex This assumption w further supported by observations that ra like positive premotor potentials have be shown in EEG in the frontal and pari areas (Becker et al 1968 Barlow & Ci neq 1969 Kurtzberg & Vaughan 1973)

Recent experiments with single unit cordings have however largely contradict these concepts Thus in single unit reco ings Bizzi (1968) Bizzi & Schiller (19 and Mohler et al (1973) were not able detect any presaccadic activity in the p frontal area although they could record a tivity during the saccade as well as dur slow eye movements



Figs 3–11 EM aspects of damaged structures in the basal coil of pup cochlea 7 days after traumatic exposure

Figs 3 4 Apical pole of OHCs. Note numerous lysosomes (*Ly*), vacuoles (*V*) and Golgi structures (*G*) 16000

Figs 5 6 IHC showing the same cytoplasmic inclusions as in OHC. Note on Fig. 5 damaged stereocilia (*arrow*) 10000 (*5*), 17000 (*6*).

Figs 7 8 Afferent fibres spiralling between Deiter' cells with vacuoles and myelin figures (*Mf*). 16000 (*7*), 9000 (*8*)

Figs 9 10 Efferent endings to OHC. Note the abnormally low density of synaptic vesicles and myelin figures (*Mf*). 15000 (*9*), 16000 (*10*)

Fig 11 Myelin figure complex (Spoendlin 1970) in supporting IHC 13000

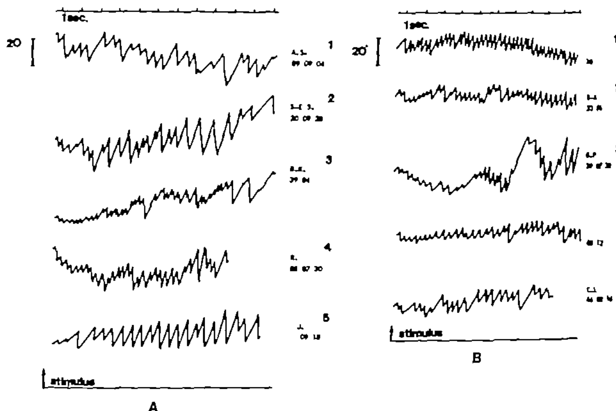


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Acta Otolaryngol 89: 323–329 1980

INVESTIGATIONS ON THE CRITICAL PERILYMPHATIC PRESSURE VALUE CAUSING ROUND WINDOW MEMBRANE RUPTURE IN ANESTHETIZED CATS

Ernő Mirisztai and Péter Sándor

From the ORL Department of the Second Pediatric Clinic and Experimental Research Department Semmelweis University Medical School, Budapest, Hungary

Abstract. Increasing perilymphatic fluid pressure was found to be an important factor in the etiology of round window membrane rupture. The critical pressure causing membrane rupture was determined in anesthetized cats. Its value was found to be in the range 10–30 mmHg ($\bar{x}\pm$S.E.M.$=23.4\pm17.1$). This value was compared with the pressure increases caused by thoracic and abdominal compression, cervical strangulation, forced Trendelenburg position, coughing and sneezing.

Although pressure and flow conditions in the perilymphatic space have been investigated from several aspects in the last couple of years, one can find scarcely any reference in the literature about the critical perilymphatic pressure values causing rupture of the round window membrane.

In the present series of experiments—carried out in anesthetized cats—we have investigated this critical range of the perilymphatic pressure.

The pressure values causing rupture of the round window membrane were compared with those increased perilymphatic pressure values which could be measured in different, experimentally induced physiologic and pathophysiologic conditions in the same experimental animals.

METHODS

The experiments were performed on 12 female cats weighing 2½–3½ kg and anesthetized with 40 mg/kg sodium pentobarbital (Nembutal, Abbott, Ottignies, Belgium) intravenously.

The trachea was cannulated with a glass cannula, and the bullae osseae were prepared free on both sides from incisions led behind the ears. The round window was exposed by opening the bulla. In one group of the animals (6 cats) a 1.5 mm hole was opened with a dental drill to the perilymphatic space through the bony wall of the round window at some millimetres distance from the window. In another group of animals (6 cats) the perilymphatic space was opened by stapedectomy.

A 1.0 mm outer diameter metal cannula was inserted into the hole and fixed with Duracryl (Duracryl "O" self-curing resin, SPOFA-Dental, Prague) dental cement. The cannula was connected to a short polyethylene tube, filled with Evans-blue stained physiological saline. The tube was connected to a three-way stopcock, the first branch of which was connected to a mercury manometer whose pressure values were recorded on a kymograph. The second branch was connected to an electric pressure transducer (P 23 AA, Statham, Hato Rey, Puerto Rico, USA) in order to have a simultaneous possibility for recording low "breakthrough" pressure values on a polygraph (Officine Galileo R 105 h, O.T.E. Biomedica, Florence, Italy). The pressure in the perilymphatic space was raised slowly and continuously through the third branch of the stopcock with the aid of a Mantoux syringe filled with Evans-blue stained physiological saline.

The experiments were carried out only in those animals in which the round window membrane was intact under the control of an operating microscope (×37 magnification).

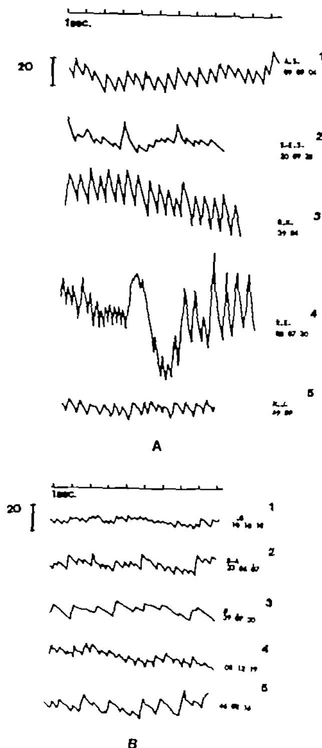


Fig 5 Nystagmus recorded after cold water (30°) irrigation of right ear (A) in patients with dyspraxia (B) in healthy controls.

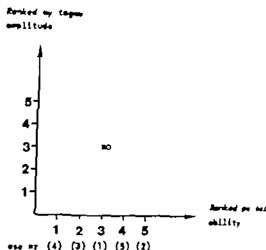


Fig 6 Ranked amplitude of caloric (x) and of optokinetic (o) nystagmus as a function of pursuit ability

in man It may be suggested that most of the eye movements studied in monkeys in laboratories were initiated by interest and the eye movements *thus excited in area* 7 (Lynch et al 1977) The command eye movements in man may be an effect of a process in frontal cortex phylogenetically newly developed speech

3 *Increase of caloric nystagmus amplitude—an effect of reduced cortical inhibition?*

An intense and rhythmic caloric nystagmus response was found in all dyspractic subjects The largest amplitudes were found when disturbances of saccades as well as pursuit eye movements indicated more extensive cortical lesions The large nystagmus amplitude may thus be an effect of reduction of cortical inhibition of a meaningless vestibular response

A careful analysis also of properties of the saccades may then be useful for evaluation of the vestibular responses and also helpful for discriminating between patients with aphasia and dyspraxia which is of interest for the phoniatrist and his selection of treatment

ZUSAMMENFASSUNG

Funf Patienten mit Sprechprogrammierungsstorungen (oraler verbaler Dyspraxie) wurden mit einer umfassenden Serie von Prüfungen der Augenmotorik untersucht Es ergaben sich hypometrische und ungenaue Saccaden mit unregelmäßigen zwischenliegenden Pausen Die Befunde deuten auf eine Ausbreitung der cortikalen Läsionen vom frontalen Abschnitt für Sprache bis zum motorischen Cortex für Augenmotorik mit daraus resultierenden Storungen der Vorprogrammierung von willkürlichen Saccaden. Diese Resultate bestatigen die

Table II *Maximum perilymphatic pressure increases (in mmHg) under the influence of test situations*

Type of the test	Cervical strangulation	Thoracic compression	Forced Trendelenburg position	Coughing	Sneezing
Number of exp animals Inner ears Measurements	3 cats 3 ears 9 cases	7 cats 13 ears 24 cases	5 cats 10 ears 22 cases	5 cats 7 ears 36 cases	5 cats 5 ears 29 cases
$\bar{x}$	1.09	0.75	1.79	1.14	1.18
S.E.M.	0.31	0.11	0.34	0.12	0.15
S.D. ±	0.93	0.53	1.58	0.72	0.80

sure changes are shown on Table II. As can be seen in this table, the most significant pressure increase was observed during the forced Trendelenburg position. In some of these cases we have observed a pressure increase of 5.8 and 4.0 mmHg magnitude, which values are close to the lowest pressure values causing round window membrane rupture in our experiments.

DISCUSSION

The symptoms of the round window membrane rupture—according to the literature data—can be observed in the form of serious disturbances of the cochlear and vestibular function and ear noises (Simmons 1968, Goodhill 1971, 1973, Pullen 1972, Freeman et al. 1974, Healy et al. 1974, Taylor & Bricknell, 1976, Allam 1976, Althaus 1977, Goodman & Morioka, 1978, Kleinfeldt 1978, Molvaer et al. 1978, Behbehani & Kastenbauer 1978).

Experimental studies published in the last couple of years have provided a considerable amount of data on the pathophysiologic background of the disfunctions (Simmons et al. 1962, Arnold & Ilberg 1972, Lamkin et al., 1975, Axelsson et al. 1977, Ivarsson & Pedersen, 1977, Weiskopf et al. 1978).

The data of Simmons (1962, 1968) and Goodhill (1971, 1973) have called attention to the significance of the perilymphatic pressure changes in the development of perilymphatic fistulas. The aim of our present investigations was to prove the connection between the perilymphatic pressure increase with the rupture of the round window membrane in experimental conditions and also to determine the pressure range of the perilymphatic fluid at which round window membrane rupture can occur. The results of the animal experiments presented show that increased perilymphatic pressure can lead definitely to rupture of the round window membrane. The average of the critical pressure values was between 20 and 30 mmHg. The highest and the lowest pressure values at which membrane rupture was observed in the experiments were 66 and 6 mmHg, respectively. In this pressure range there was no accompanying oval window membrane rupture. This fact makes it certain that the tissue structure of the oval window membrane is more resistant to the increased pressure than is that of the round window membrane, in spite of the observation that the tissue layer covering the perilymphatic space from inside appears to be a homogenous arachnoid structure (Franke 1979).

According to our present results, the pressure values causing rupture of the round window membrane can show considerable individual differences, and significant differences were found in this respect even in the two inner ears of the same animal. We assume that this phenomenon is probably due to the structural and mechanical resistance differences of the membranes. The perilymphatic pressure

Acta Otolaryngol 89 152–156 1980

THE ORIGIN OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) NERVES IN THE FELINE NASAL MUCOSA

R. Uddman L Malm and F Sundler

From the Department of Otolaryngology University Hospital Malmö and the Department of Histology University of Lund Lund Sweden

(Received April 4 1979)

Abstract The feline nasal mucosa is richly supplied with vasoactive intestinal polypeptide (VIP) immunoreactive nerve fibres The nerves occur in the subepithelial connective tissue around small blood vessels and around the acini of seromucous glands The pterygopalatine ganglion contains numerous VIP immunoreactive nerve cell bodies among non-reactive ones Extirpation of the ganglion results in an almost complete disappearance of VIP-containing nerves in the nasal mucosa, while sectioning of the preganglionic nerve (Vidian nerve) does not affect the number of nasal VIP nerves. Hence the bulk of VIP nerve fibers in the nasal mucosa derive from cell bodies located in the pterygopalatine ganglion

Many electrophysiological pharmacological and ultrastructural investigations point to the existence of non adrenergic non-cholinergic nerves in various tissues (Burnstock 1975) The atropine resistant vasodilatation in the nasal mucosa that results from electrical stimulation of Vidian nerve may indicate the occurrence of such nerves (Malm 1973*a* Ånggård 1974) One obvious candidate for this type of nerves is the system of vasoactive intestinal polypeptide (VIP) containing nerves Several recent studies have shown VIP nerves to be widely distributed in the body (Bryant et al 1976 Larsson et al 1976 Alm et al 1977) There is accumulating evidence that VIP may act as neurotransmitter or modulator (Giachetti et al 1977 Emson et al 1978) We have previously reported on the presence of a rich number of VIP nerves in the upper respiratory tract of several mammals (Uddman et al 1978 1979) In these studies we found evidence for a local orogin of tracheo-bronchial VIP nerves since VIP-containing nerve cell bodies were regular constituents of intramural ganglia. In the nasal mucosa on the other hand no such cell bodies were detected We now wish to present evidence that the VIP nerves innervating the nasal mucosa originate in the pterygopalatine ganglion

MATERIAL AND METHODS

Thirteen cats of either sex were anaesthetized by intracardial injections of sodium pentobarbitone after induction with diethylether In 1[illegible] of the cats the pterygopalatine fossa on one side was exposed through a transpalatal approach (Malm 1973*b*) and the pterygopalatine ganglion a proximal segment of the posterior nasal nerve and a distal segment of the Vidian nerve extirpated and processed for immunohistochemistry (see below) Two of the denervated cats were killed two days after the operation and nine cats one to three weeks after the operation by exsanguination during pentobarbitone anaesthesia In the remaining two cats unilateral extirpation of a short segment of the Vidian nerve was performed during shortlasting anaesthesia These cats were killed three weeks after the operation In all cats specimens were taken from various locations in the nasal mucosa (septum maxilloturbinal ethmoturbinal and nasoturbinal area

Grant support was received from the Swedish Medical Research Council (04X [illegible])



Abb. 1 (*a*) Zusammengesetzter Ausschnitt aus der Stria vascularis des *Falles 1*) Erste Windung: *P* Prominentia spiralis, *E*, flaches Recessepithel; darunter unmittelbar angrenzend Ligamentum spirale (*L*). Im oberen Anteil besteht noch ein massives Striaödem (↗). Insgesamt aufgehobene Striamorphologie und Fehlen sämtlicher Kapillaren. 460. (*b*) Zweite Windung: über der gesamten Striaausbreitung schweres Ödem mit Verlust aller charakteristischer Strukturen. 460

den Befunden bei der experimentellen Masugi-Nephritis den Einfluß von Nierenerkrankungen *auf das Innenohr zu erhellen.*

MATERIAL UND METHODIK

Die Felsenbeine zweier Patientinnen mit Alport Syndrom wurden in einem Falle 1,5 Stunden post mortem, im anderen Falle 45 Minuten post mortem transmeatal nach Herausnahme des Trommelfelles, des Steigbügels und nach Eröffnen des runden Fensters durch Perfusion mit 3,5%igem eisgekühltem Glutaraldehyd perfundiert. Nach der Sektion

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Für hervorragende technische Mitarbeit danke ich Frl. S. Linnenkohl.



Fig 3 Pterygopalatine ganglion. Numerous VIP-containing nerve cell bodies throughout the ganglion Note also the presence of immunoreactive nerve fibres among the cell bodies (×150)

epithelium around blood vessels and around the acini of seromucous glands (Fig 1)

The posterior nasal nerve contained a large number of coarse VIP immunoreactive nerve fibres (Fig 2) Occasionally VIP immunoreactive nerve cell bodies were noted within the most proximal segment of the nerve trunk

The pterygopalatine ganglion contained numerous fairly large cell bodies displaying a varying degree of VIP immunoreactivity dispersed among non reactive ones (Fig 3) Immunoreactive nerve fibres were regularly found within the ganglion In the most distal segment of the Vidian nerve adjacent to the ganglion a few VIP immunoreactive nerve cell bodies and nerve fibres were sometimes found More proximal segments of the nerve were devoid of VIP immunoreactivity Unilateral extirpation of the ptery ganglion *resulted in an almost com* appearance of VIP nerves in the nas of the same side as studied one to th after the operation (Fig 4*a*) The r VIP nerves in the contralateral sid visibly affected (Fig 4*b*) Two days turpation of the ganglion a rich numb fibres could still be observed in mucosa Unilateral extirpation of o *ment of the Vidian nerve did not se* fect the number of immunoreactive ves in the nasal mucosa.

DISCUSSION

The nasal mucosa has previously bee to contain an extensive supply of a and acetylcholinesterase positive *forming plexa in subepithelial conne* sue around small blood vessels a glands (Dahlström & Fuxe 1965 Ish Änggård & Densert 1974 Grote et al The adrenergic nerves to the nasal seem to have their origin in the supe vical ganglia (Änggård and Densert Grote et al 1975) while the cholinergi reaching the mucosa seem to emana the pterygopalatine ganglion (Grote 1975)

The present results on the occurre distribution of VIP immunoreactive ne the feline nasal mucosa confirm previo ported findings (Uddman et al 197 VIP fibres were distributed in the subep connective tissue around blood vess around the nasal glands VIP is a poten dilatory agent (Said & Mutt 1970) and known to stimulate the secretory acti various exocrine glands (Said & Mutt Konturek et al 1976) It is therefore co able that nasal VIP nerves are involved regulation of local blood flow and of mucous secretion

A rich number of coarse VIP nerve found in the nerve distal to the pterygopa ganglion (posterior nasal nerve) The

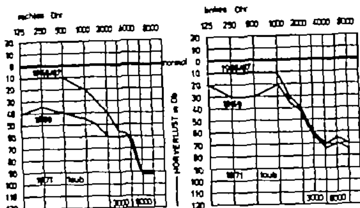


Abb 4 Tonschwellenaudiogramme 1966, 1969 und 1971 In den letzten Lebensmonaten war die Patientin taub

der Stria vascularis, aber nur eine geringfügige Degeneration der Prominentia spiralis. Lediglich im Bereich der dritten Windung fanden sich noch Bezirke von epithelähnlichem Aufbau mit weitgestellten Gefäßen Der Flüssigkeitsraum des nur mehr spärlich vorhandenen Ligamentum spirale erstreckte sich bis unmittelbar unter die Epithelreste (Abb 1*a b*) Die Reissner sche Membran war intakt Im Bereich des Limbus spiralis imponierte ein Kondensat von PAS-positivem Material an der Oberfläche der Interdentalzellen Auch die Tectorialmembran färbte sich intensiv PAS-positiv in ihren Randbezirken an Das Corti sche Organ wies in allen Windungsanteilen einen vollständigen Verlust der inneren und äußeren Haarzellen auf die Tunnelradialfasern fehlten in den Serienschnitten (Abb 2) Das Ganglion spirale ließ eine Degeneration der Nervenzellen erkennen wobei die umhüllenden Mantelzellen erhalten blieben und somit häufig lochförmige Areale zu erkennen waren (Abb 3) Tonaudiometrisch bestand bei dieser 38-jährigen Patientin zum Todeszeitpunkt eine beidseitige Taubheit (Abb 4)

Fall Nr 2

Bei dieser 42-jährigen Patientin mit familiärem Alport Syndrom war mehrere Jahre vor dem Tod durch eine Nierenbiopsie, durch die klinische Symptomatik einschließlich eines geringfügig zunehmenden Hochtonverlustes und einer Linsentrübung die Diagnose bestätigt worden Die Patientin verstarb im Zustand der kompensierten Niereninsuffizienz an einem akuten Herzinfarkt Sie erhielt während der gesamten Erkrankungsjahre keine Innenohrtoxischespharmaka Die Nierenbiopsie 1974 ließ im Bereich der Nierenglomerula noch eine Verbreiterung der Basalmembranen sowie eine Verschmelzung der epithelialen Zellausläufer erkennen die nach David et al (1966) und nach Zollinger & Mihatsch (1978) kennzeichnend für glomeruläre Veränderungen beim Alport Syndrom sind (Abb 5*a b*) Zum Zeitpunkt dieser Nierenbiopsie wies die Patientin eine äußerst geringgradige vorwiegend im Hochtonbereich gelagerte Innenohrschwerhörigkeit mit deutlichem Seitenunterschied auf In den folgenden Jahren verschlechterte sich die Nierensituation und gleichzeitig kam es von einer geringgradigen Schwerhörigkeit zu einer mittelgradigen wiederum seitenunterschiedlichen Schwerhörigkeit, dabei war die stärkste Zunahme des Hörverlustes im Hochtonbereich vorhanden (Abb 6)

Die Untersuchung der Nierenglomerula zum Todeszeitpunkt ließ eine weitgehende Degeneration und Obliteration erkennen elektronenmikroskopisch waren die meisten Gefäße durch eine enorme Proliferation der Epithelien und Verdickung der Basalmembranen eingeengt oder obliteriert (Abb 7)

Die Stria vascularis war in allen Windungsanteilen voll erhalten, ausschließlich im Bereich der Reissner'schen Membran und unmittelbar

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Rolf Uddman M.D
University of Lund
ENT-department
Malmo General Hospital
S 214 01 Malmö
Sweden



Abb. 9(*a*) Corti-Organ der zweiten Windung. Degenerative Veränderungen der Inneren Haarzelle (*IHZ*) bei normaler Struktur der Äußeren Haarzellen (*ÄHZ*). ×485. (*b*) Corti-Organ der Basalwindung. Äußere Haarzellen intakt, Innere Haarzelle pyknotisch. (↗) Sinneshaare einer *Äußeren Haarzelle haften an Tectorialmembran.* ×485



Abb. 10. Ausschnitt aus dem Ganglion *spirale der Basalwindung.* Normale Struktur. Fehlende Myelinisation ist nicht pathologisch. ×493

Almqvist & Wiksell Uppsala 1980

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W. Arnold, M.D.
Hals-Nasen-Ohrenklinik
der Universität Düsseldorf
D-4000 Düsseldorf
Moorenstraße 5
Deutschland

DISCUSSION

Kelemen to Arnold: Emphasizes scepticism regarding the dilatation theory in Meniere and other conditions.

Temporal bone histology requires an equally careful preparation whether for light or electron microscopy.

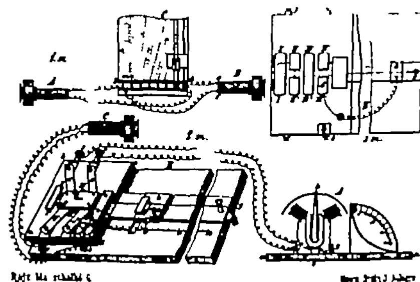


Fig 3 Telephone-like equipment for objective measurement of hearing.

president and organizer of the Collegium meeting here in Budapest

In brief I should like to mention a few data from the history of Hungarian Otolaryngology

Endre Hőgyes was professor of pathophysiology in Kolozsvár and in Budapest (Fig 1) His life extended from 1847 to 1906 In 1880 the associated movement of eyes and their connection with the actions of the semicircular canals was investigated and described by Hőggyes He performed the first experiments for objective measurement of hearing by means of a telephone-like electric apparatus (Figs 2 and 3)

Jan Czermák was professor of physiology in Krakow in Budapest and later in Prague (Fig 4) He was not a Hungarian and he did not speak in our language But during his stay in Budapest the examination of larynx by a mirror was discovered by him and also the examination of the epipharynx by means of this mirror (Fig 5) The first photograph of the larynx was also taken by Czermák

Not far from Budapest in Albertirsa Ádám Politzer was born in 1835 (Fig 6) Today we can still find a memorial tablet on his house where he was born This tablet was placed by the Hungarian and Austrian Societies. Politzer s scientific activity in otology first of all in histopathology of the ear is well known The Politzer balloon is used all over the world

The next great name which must be mentioned is Robert Bárány The Bárány family lived in Rohonc but his parents left Hungary for Vienna

Bárány s life and scientific works are all well known from their beginning to the N



Fig 4 J Czermak

Table I *Summary statistics for puretone AC thresholds*

Frequency (kHz)	Right ear				Left ear			
	N	Mean	Median	S.D	*N*	Mean	Median	S.D
0.5	1 203	25.2	22.7	13.[illegible]	1 203	25.8	23.5	13.4
1.0	1 206	36.3	36.3	15.2	1 204	36.9	37.7	15.8
2.0	1 204	52.1	53.6	14.3	1 203	54.1	55.4	13.9
3.0	1 203	6[illegible].9	63.4	13.8	1 198	64.2	64.4	13.[illegible]
4.0	1 195	68.3	68.1	14.4	1 195	69.2	68.6	13.6
8.0	1 184	67.[illegible]	68.2	20.2	1 182	68.5	69.4	19.1

is that the reflex plane is divided by two intersecting line segments apparently the slope of the line segments and the point of intersection are determined by inspection not by any formal optimisation method. This makes statistical interpretation difficult.

Johnsen et al (1976) stated that ARTs for puretones in patients with cochlear deficits are largely independent of the audiometric threshold, and they queried the logic behind the Niemeyer Sesterhenn prediction formula. However they showed a clear positive association between average hearing loss and the ART for white noise but they did not express this in terms of a linear regression equation. They showed a scattergram for hearing loss against the difference between puretones and white noise ARTs which showed very little association. It may therefore be inferred that inclusion of the puretone average ART in a prediction equation may not contribute to the prediction accuracy and may even degrade it.

Keith (1977) reviewed the work of Niemeyer & Sesterhenn and Jerger et al noting that audiogram slope prediction by reflex methods is equivocal and that prediction methods based on white noise reflexes were as accurate as those which also included puretone ARTs.

Hall (1978) compared two versions of the Jerger predictor with an unpublished regression predictor reported by Baker & Lilly (1976). The predictive accuracy of the three methods was similar and decreased systematically as a function of increasing age. The performance of the Jerger predictors was degraded in the presence of minor abnormalities of the tympanogram.

In a comprehensive report Jerger et al (1978*a*) examined the effect of age degree of hearing loss and audiogram configuration upon the ARTs to puretone and wide-band stimuli. They found that all three variables influenced the reflex relations and that their effects were interactive. Jerger et al (1978*b*) reported the effects of age on reflex prediction. They found that predictive accuracy was substantially better in a group of children than for either young or old adult groups.

Present study

We attempted to model existing and new schemes in order to evaluate the efficacy of ARTs in hearing threshold determination of a specific population—adults with presumed noise-induced hearing loss (NIHL). From this group 1 207 patients referred to our clinic (MSH) for assessment between January 1975 and January 1978 with reliable puretone audiograms and bilateral reflexes to wideband noise were selected as subjects. Data were analysed on the University of Toronto IBM 3033 computer primarily using the Statistical Package for the Social Sciences (SPSS).

Reflex measurement methods. The ART measurements considered here were obtained using standard clinical methods. Contralateral ARTs were obtained to tonal stimuli at frequencies of 250 500 1 000 2 000 3 000 and 4 000 Hz, and to unfiltered low pass and high pass filtered white noise. The 3 dB point was 600 Hz for both filters. The units for the tonal

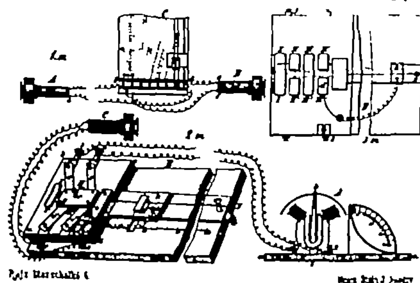


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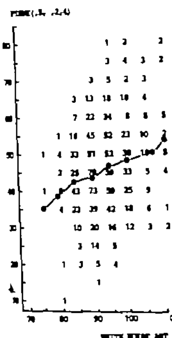


Fig 3 Cross-tabulation of average ART at 0.5 1 2 and 4 kHz and the white noise ART Circles are the conditional mean average PTT

Table VI *Conditional statistics analysis of variance and regression data for the average PT threshold at 0.5 1 2 and 4 kH by ARTWN*

PTAVE statistics

ART value	Mean	S D	*N*
75	34 4	1..5	6
80	39.0	10 7	23
85	42.8	9 1	165
90	43.8	10.3	357
95	46.3	10.6	350
100	47.9	11.0	186
105	49.5	10.5	80
110	53.9	14.2	25

Analysis of variance:
Between groups. p less than 0 0001
Linearity p less than 0 0001
Nonlinearity NS

Simple linear regression:
PTAVE=7 6+0.4 ARTWN

Standard error of slope: 0.048.
Residual mean square: 109 7
F ratio 72.9 p less than 0 0001
Number of cases: 1192.

and thereafter shows a rapid rise The white noise ART increases fairly linearly throughout the loss range The convergence principle is therefore valid for losses up to 70 dB These results are comparable with those obtained by Jerger et al. (1978*a*)

(*e*) *Prediction of audiogram features from ART measurements* Most published reports on prediction of hearing level from ART measurements consider average hearing losses Such an approach arises from an historical perspective or because of an underlying presumption that average hearing losses will somehow relate better to ARTs than will single puretone hearing loss measurements The results in Table V show that this is not entirely true but the noise ART/average loss correlations are among the highest in the table

The predictability of the average puretone loss at 0.5 1 and 4 kHz by single ARTs was examined From Table V it can be seen that the white noise and high pass noise ARTs correlate equally well The former was selected for detailed study because of its higher incidence (Table II)

The cross-tabulation of the average behavioural threshold at 0.5 1 2 and 4 kHz versus the white noise ART is replotted in Fig. 3 with the white noise ART as the abscissa. It can be seen from Fig 3 that the conditional mean average loss increases linearly with increased ART (conditional statistics and regression data are given in Table VI) The key observation is that the range of the conditional means and therefore the approximate range of prediction, is only about 20 dB and the standard error of prediction is greater than 10 dB at any value of white noise ART Therefore although the white noise ART does permit prediction of average hearing losses, the clinical utility of this prediction is extremely limited

Clearly the conditional average loss distributions will depend upon the population under study If this were restricted in range then the range of prediction would also be restricted We do not consider this to be a serious objection to these data. The range of average losses

years ago (Fig 7) His findings in the physiology of hearing are well known since they became public property

I should now like to say a few words about the present situation of ORL in Hungary At present we have four universities for medical students and one postgraduate medical school also with an ENT department for doctors and specialists

Today there are in Hungary more than 450 ENT specialists among the 23 500 physicians

To round off my opening speech I have the honour of mentioning that the oldest member of our Collegium is Gy Keleman as I know he is now 90 years old Let me convey to him from my heart—in the name of the Collegium membership and also of all Hungarian oto-laryngologists—our best respect and gratitude

L. Surján

P O B 112
H 1289 Budapest
Hungary

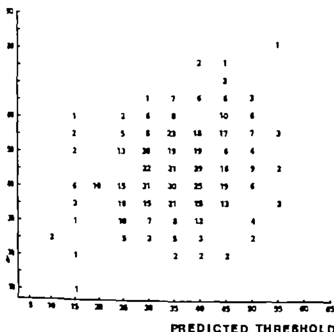


Fig 5 Cross-tabulation of observed average PTT (ordinate) predicted average PTT (abscissa) from the Niemeyer Sesterhenn predictor

Comparative assessment of predictive formulae

The predictive performance of the Niemeyer–Sesterhenn formula was compared with prediction using white noise alone and with a multiple regression equation derived in this study The Niemeyer–Sesterhenn formula incorporates the 4 kHz ART Accordingly the formulae were compared in a sub-population of 719 patients having all appropriate reflexes

In Fig. 4 the difference between the average ART at 0.5 1 2 and 4 kHz and the ART for white noise is cross-tabulated against the average ART minus the average puretone threshold at those requencies This corresponds to Fig. 3 in Niemeyer & Sesterhenn s original report (1974)

The solid line in Fig. 4 corresponds to their prediction equation which states that the abscissa is 2.5 times the ordinate The data of this study do not follow that relationship the bivariate distribution is clearly more horizontally oriented than that required by the formula. A substantial number of points are outside the 20 dB limits especially for small values of the abscissa.

One way of visually assessing the performance of a prediction equation is to cross-tabulate the observed and predicted values In Fig 5 this coss-tabulation is shown for the Niemeyer–Sesterhenn predictor If the predictions were perfectly accurate all the points would lie on a diagonal line with unit slope The enormous scatter is clear from the figure There is a tendency for predictive errors to be positive at low predicted values and negative at high predicted values As well as a great deal of variation there appears to be systematic bias in prediction These results differ in both location and scatter from those of Miller et al. (1976) but that study is both small-sample and contains a normal-hearing group

Carrying out a simple linear regression of the four-frequency average hearing loss on the white noise ART in this sub-population gave a predictive formula shown in Table VII the correlation coefficient was 0.22 The cross-tabulation of observed and predicted values is

lms with the complement system etc but the acute or chronic inflammation is not enough to investigate alone by the methods of classical immunology

(4) In our field some misinterpretation is also observable The immunological reactions or other factors of the defence mechanism are neglected or on the contrary overestimated e g in cases of acute middle-ear inflammation or in indication of tonsillectomy

(5) Very serious problems arise from the fact that our minds are far more influenced—as was mentioned above—by the good or appar ently good effect of antibiotics than by rea knowledge of the defence reactions of th body and very often we work against them For instance the defence mechanisms are ver frequently inhibited by the antibiotics This i observable first of all if antibiotics are admini tered too early in the inflammation

I believe that the above-mentioned prob lems are very important Therefore it woul seem to be very useful to discuss these com mon problems of immunology and oto-laryn gology

necessary to achieve this involve completely different sets of variables.

The decreasing effectiveness of the white noise reflex as a predictor of average hearing loss in the high slope group and the production of completely different multiple regression predictor equations for the low slope and high slope groups, make the use of ART prediction procedures even more problematic

One possible solution to this is to develop prediction procedures in two stages the first consisting of the prediction of slope and the second a prediction of severity To this end the ability of reflex measures to predict audiogram slope was examined The measure selected was the ART to high pass noise minus the ART to low pass noise This was both correlated and cross-tabulated against the observed audiogram slope as measured by the difference between the 4 kHz and the 1 kHz puretone thresholds The correlation coefficient obtained was only 0 22 and the cross-tabulation showed no appreciable diagonalization. It is concluded that this ART measure cannot serve as a useful basis in a multistage prediction process

One final and simple examination undertaken attempted to relate values of PTT to ARTs for PTs It has often been pointed out that if a recorded PTT were worse than a measured ART for that frequency the PTT must be in error (Alberti 1970) However the literature is not clear how much higher the ART must be than the PTT before the latter can be considered reliable We investigated what level of PTT had a less than 10% chance of actually being exceeded for different decade levels of ART The results for 2 kHz are shown in Fig. 8 where it can be seen that if the ART is 100 dB or greater the PTT has a 90% probability of being at least 35 dB better whereas if the ART is 90 or 80 then the PTT may come to within 25 dB of the ART It it comes closer the patient should be re examined audiometrically before accepting the result

The results of this study are disappointing. In adults objective predictive techniques are potentially of greatest use in medico-legal settings, particularly with large populations such as in screening industrial hearing loss claims It is exactly such a population which has been tested and where all simple techniques appear to have no real quantitative value ART measurement has a qualitative role to play both in site of lesion testing and in identification of hearing loss but no accurate quantitative predictions about hearing loss can be based upon it

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Dr P W Alberti
Suite 403 600 University A enue
Toronto Ontario
Canada M5G 1X5

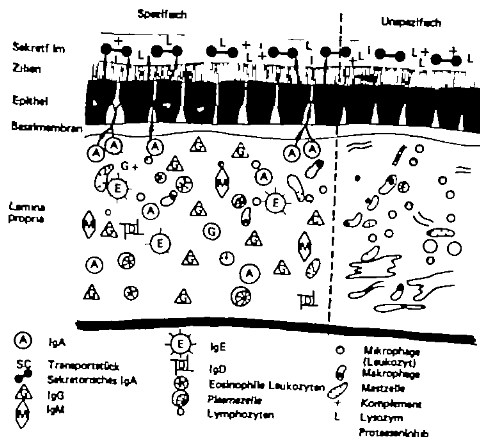


Fig 1 Synopsis of some s cific and non-specific fact influencing the defense mechanism of the respirat mucosa

Lysozyme is the longest known protective enzyme in nasal secretion It attacks glucoside compounds in polysaccharides and mucopeptides such as occur in the cell wall of Gram positive bacteria The action of lysozyme causes the step-by step disintegration of the bacterial cell walls—probably due to the simultaneous action of complement (Glynn 1966)

Gram-negative bacteria incidentally are protected from lysozyme attack by a lipopolysaccharide component in their capsules (Mims) According to recent studies lysozyme which is bacteriostatically bactericidally and bacteriolytically active is produced among other things in the mucosa glands It can also be detected in large amounts in the police cells (macrophages and microphages) There are indications that lysozyme has a synergistic effect not only with complement but also with immunoglobulin A

Yet another aspect is of interest in this context: the lysis of the bacterial cell wall caused by lysozyme is not carried down to the monosaccharide stage but only to the larger decom position complexes (penta-*N*-acetylglucos minide) Further decomposition of the ce wall fragments including complete hydroly is then carried out by various glucosidase which are also present in the nasal secretion This is important because cell wall substanc which is only partially decomposed is highl immunogenic

Lactoferrin also produced in the respirato ry mucosa and also detected in neutrophilic leukocytes has a protective function agains microorganisms as well In vitro it apparently inhibits the growth of bacteria such as *Staphylococcus albus Staphylococcus aureus* and *Pseudomonas aeruginosa* by removing iron ions from the nutrient medium

The lysozyme group acts against bacteria, but not viruses Interferon by contrast also present in nasal secretion is characterized by considerable antiviral activity This protective protein is formed by very many cell types—including epithelial cells—within a few hours

2) The analysis of 25000 tone threshold audiograms made after hearing losses and noise exposure confirms the data gained in practical work.

3) Experimental studies show—as a consequence of the 85 dB(A) noise level—a lack of hearing recovery which is the ultimate cause of the permanent hearing defect

ZUSAMMENFASSUNG

Wie die jahrzehntelange ärztliche Begutachtung zeigt, bestehen stärkere Hörschädigungsgefahren nicht erst ab 90, sondern schon ab einem Lärmpegel von 85 dB(A). Diese Erfahrung wird durch eine Analyse von 25000 Tonschwellenaudiogrammen und durch das Ergebnis experimenteller Gehörermüdungstest bestätigt.

F. Schw ertz
I HNO-Univ.Klinik, Wien
A-1090 Lazarettgasse 14
Austria

DISCUSSION

Spoendlin to Schw ertz: Your first slide showed that there very little barely significant increase of damage risk between noise levels of 85 and 90 dB(A) whereas much greater increases exist between 75 and 80 dB(A) or 90 and 95 dB(A) What therefore is the reason t propose 85 dB(A) as permitted noise level limit rather than 90 dB(A) as it is in Germany and Switzerland? The barely significant increase in damage risk will probably not justify the enormous costs of reducing industrial noise emission by 5 dB

Tonndorf to Schw ertz: W in the US would also be happier with 85 dB(A) maximal level However as you are fully aware this is not a scientific but a political decision At the international level at the ISO (w ll have meeting in Delphi? Sept. 26) I am happy to report (1) that the new standard will no longer give fixed values like 90 dB(A), but only guidelines so as to force the administrative authorities and into action, (2) the three speed frequencies will be increased to *four* 5 1.0 2.0, 3.0 HHZ which will make the index more sensitive

Schw tz (Reply):

The contributions made to the discussion, for which I am most grateful can be answered together

Of course the problem of fixing the critical intensity value at certain point is also a political question in that it touches pon the financial aspect But as it involves the question of maintaining the health of the working population, it must be seen first and foremost as medical one I too encountered difficulties with the competent authorities whe many years ago I wanted to introduce 85 dB(A) as the critical intensity value to be generally observed in Austria what I demanded at that *time was based only on individual observations! The* results which are now submitted should make *it easier* for the medical profession to convince the politicians through factual material

As concerns the effect of noise-induced damage on sound-hearing, let me say the following: For the maintenance of speech hearing neither the auditory threshold for 3 kHz nor that *for 2 kHz is of any decisive significance*. As I was able to show only recently on the basis of larger number of patients examined, the speech discrimination capacity depend above all on the auditory threshold loss at 1.5 kHz in the case of typical noise-induced damage

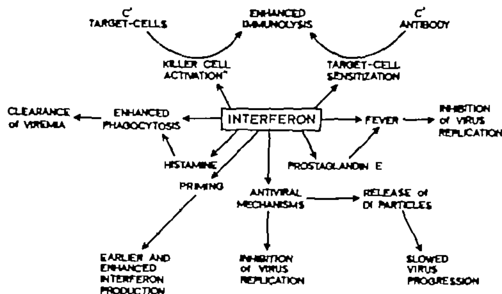


Fig 4 Involvement of interferon in host defense mechanisms (from W E. Stewart II)

This is one of the most important unspecific biological effector systems It also acts as a mediator in immune reactions and intensifies them (Fig 5) Complement is an enzyme system consisting of nine individual factors whose individual components are present as inactive precursors in blood tissue and secretions The complement system can be activated by immuno-unspecific and by immuno-specific mechanisms It then proceeds similar to a chain reaction which passes either via the classical path (C 1–C 9) or by a shortcut, the alternate path beginning at C 3 resulting in inflammation and cell destruction The so-called C 3 shunt is triggered by the properdin system (serum protein plus complement plus Mg^{++}) which is directed against Gram-negative bacteria and a few types of virus (Schmidt & Heide 1970) Without being able to go into further detail it should be mentioned that complement fulfils four anti microbial functions in the defense of infections (Mims): (*a*) activation of the inflammation reaction (*b*) attraction of leukocytes and plasma factors to the site of conflict with the invader (including the release of anaphylatoxin and the resultant degranulation of the mast cells) (*c*) activation of phagocytosis and (*d*) destruction of micro-organisms and/or contaminated cells

The aim of the complement system is to make holes in or destroy the membranes of the foreign cells This is due to an interaction of the component constituents C5–C9 with specific phospholipids in the cell membrane

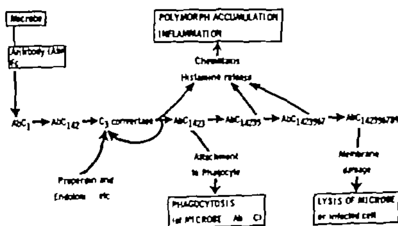


Fig 5 Complement activation sequence and antimicrobial actions (from C A Mims)

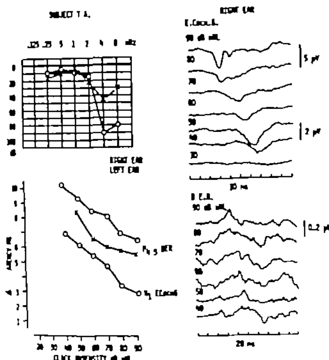


Fig. 3. Typical example of the effects of serious high-frequency hearing loss on BER responses. Compare the results with those in Fig. 2.

measures to latencies. In Fig. 1 are presented the results from one normal subject. In all normal cases the latency/intensity curves of ECochG peak (N) and BER wave P_{4-6} were parallel and showed a latency difference of about 4 ms (see also Fig. 5). At high intensity levels the latency of the first peak (P) in BER could be measured and was found to correspond to that of ECochG N but for middle and low intensities it was impossible to detect.



Fig. 4. Result from Menière patient presenting with recruitment. Although the threshold is elevated, the first peak (P) in BER recordings appears clearly.

Cochlear/retrocochlear cases

On the basis of ECochG results alone we found that the strongest indicator of a retrocochlear disorder was a threshold level better than could be expected from the subjective hearing revealed by the audiogram. Statistical studies relating the click threshold at ECochG to the subjective audiogram showed that it is a good indicator of subjective audiometric thresholds for middle frequencies (Eggermont, 1976). In all the subjects in this study we compared the ECochG click threshold with the best audiometric threshold for frequencies of 1, 2 and 4 kHz. The ECochG click threshold was better in 4 out of the 14 patients with a confirmed tumour; such a phenomenon was never observed in the group with cochlear impairment or in the normals. The pattern of ECochG response in itself did not appear sig

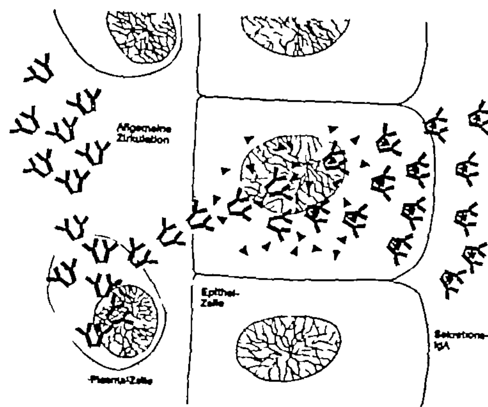


Fig 8 Scheme showing production of IgA and its synthesis to secretory IgA (from O G Bier).

the invader is immunospecific or -unspecific Mention of this group of agents brings me to a special field with which Prof Hochstrasser and his working group at our Munich clinic are involved Nevertheless the explanation will be very brief

The protease inhibitors are themselves proteins which due to their structure can interact with various proteolytic enzymes to form complexes They constitute a type of regulator for enzymatic activity In the case of humoral

BARRIER

EPITHELIUM AND BASAL MEMBRANE

I CILIA

II EPITHELIAL FORMATION

III BASAL MEMBRANE

IV CRITICAL THRESHOLD OF RESORPTION

Fig 9

and cell linked immune reactions the result is cell destruction together with an associated release and activation of protein-splitting enzymes the proteases If the proteases could act in an unchecked manner the infected organism would be partially digested at the site of the conflict and thus damaged The protease inhibitors prevent this from happening

According to the current stage of research three groups of protease inhibitors can be differentiated

(1) High molecular acid instable inhibitors (Serum-inhibitors) present substantially in the circulatory system and the interstitium. They probably pass over into the secretion only by transudation

(2) Another inhibitor group can pass through tissue and is secreted easily It is found in many mucous membranes These low-molecular inhibitors prevent mucosa damage by proteases from the body s own cells as well as to proteases released directly and indirectly by microorganisms They are acid-proof and make up approximately 80% of the anti tryptic activity of the secretion The precursors

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M Portmann
Clinique Universitaire d'O R.L.
Laboratoire d'Audiologie Expérimentale
(F.R.A Inserm no 39)
Université Bordeaux II
Hôpital Pellegrin
Place Amélie-Raba-Léon
33076-Bordeaux Cedex
France

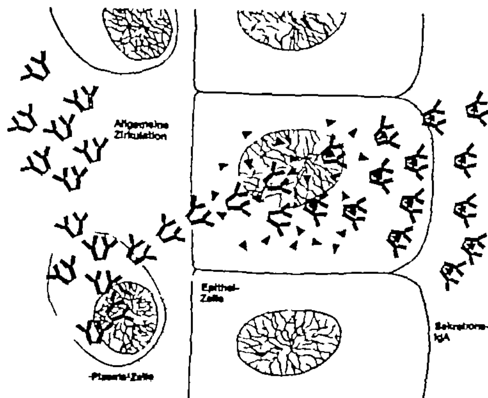


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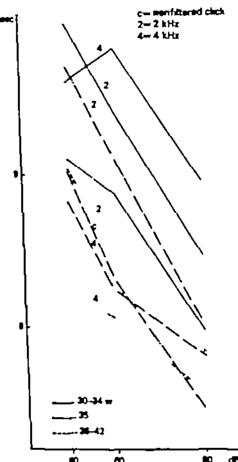


Fig 1 Latencies J in function of gestational age

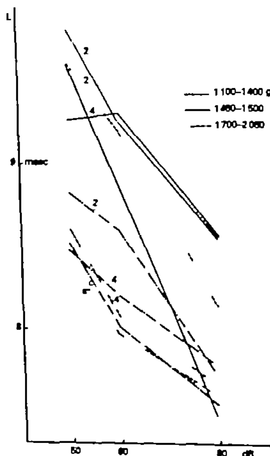


Fig 2 Latencies J in function of weight.

Respective influence of age and weight J wave latency for non-filtered clicks was compared in the four conditions where the variables were as follows gestational age alone weight alone weight and age in two groups weight and age in three groups

Gestational age proved to be the most important factor but the influence of weight was greater for the lower weights and with lower intensity stimulation, that is 60 and better still 50 dB

Little opportunity to observe pathological recordings

Our experience was limited to one case of a full-term baby weighing 3 300 g admitted to hospital with pyocyanic septicaemia treated with amikacin This baby died The B S R.A recording performed a week before death was completely flat

A single extended study in ideal conditions of one baby examined three times

1 200 g, 35th week
1 500 g 38th week
2 050 g, 42nd week

The quality of the recordings made it possible to measure the latencies of J J_{III} J and J_{VI} waves. Latency at 2 050 g reached at the 42nd week full-term, was not the same as for an adult but AP-J_V was close to it.

CRITIQUE

The major criticism of the present study is the limited number of observations made In fact

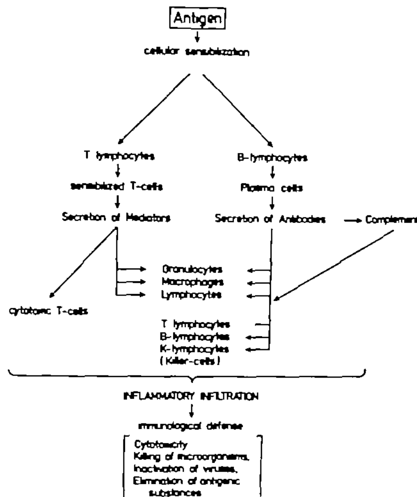


Fig 11 Functional relations between B cells and T cells (from K Resch)

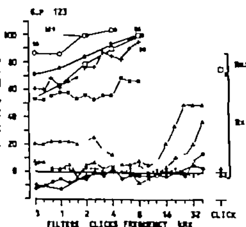
This immune body can also be formed in the mucosa but acts as the very largest of all immunoglobulins especially intravascularly

Only with elevated vascular permeability does IgM penetrate into the tissue and thus supplement the IgM concentration which may already be present there It is found only in trace amounts in nasal secretion Only in individuals who cannot produce any IgA can it take over its place and function in the secretion and mucosa area

In the first line of defense IgA is undoubtedly the most interesting and most important immune body Owing to its combination with the secretion it is also termed a muco-antibody which together with the secretion layer forms an antiseptic protective film

IgA is the immunoglobulin of the mucous membranes and has the task of preventing accumulation adhesion and invasion of micro-organisms and other antigens on and in the mucosa Unlike other immunoglobulins it requires a relatively complicated composition to be able to perform its protective function on the mucosal surface A monomeric form of IgA is formed in the plasma cells especially in the mucosa The monomers join together to form di and polymers in the lamina propria of the mucosa and are then transported in the direction of the mucosa surface in particular adjacent to the mucosal glands (Fig 8)

A so-called secretory piece which is produced in the epithelial cells (Tomasi 1976) must appear however to activate the surface protective function Only the combination of the polymeric IgA with the secretory piece form the secretory IgA which is active on the mucosa surface or in the secretion The secretory piece apparently facilitates the passage of the IgA polymer through the epithelium and



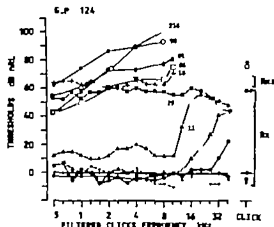


Fig. 1. Frequency threshold curves of the responses recorded at the round window for guinea pigs at various times indicated in days (*small characters*) after the beginning of the first amikacin treatment (day 0). Note the rapid evolution during the first treatment (Rx1: 14 450 mg/kg/day) and the small changes during and after the second treatment (Rx2: 20 doses), particularly for GP 124 (*right*).

while the other ear, not involved by the procedure, served as a control at time of sacrifice of the animal. The surgical procedure as well as the method of stimulating and recording from this electrode in awake animals have been described in detail elswhere (Aran & Erre 1979) and used in various studies (Aran & Darrouzet, 1975; Aran & Cazals, 1978). As usual, the auditory nerve compound action potential (CAP) thresholds to filtered clicks from 0.5 to 40 kHz are determined (CAP audiograms), the intensity levels being referred to the mean threshold of normal responses (dB nRL). Although we normally use a Bruel & Kjær condenser microphone cartridge (4134) directly coupled to the ear as a transducer, in these experiments as soon as the thresholds became elevated we had to use a TDH 39 Telephonics earphone in order to obtain higher sound levels, although the frequency of the transient stimuli could not then exceed 10 kHz. Responses to the unfiltered click using this earphone were also recorded from the very beginning of the experiments so that patterns of the responses and input–output amplitude and latency functions could be monitored over the entire experiment. As usual for the recordings w[illegible]matically used averaging and clicks of alternate polarity to cancel out any microphonic potential.

Effects of the first treatment

These 5 implanted guinea pigs, having normal click-evoked responses and CAP audiograms, received a first treatment of amikacin identical with that used in our earlier studies (Aran et al. 1979; Cazals et al. 1979*b*), i.e. 14 intramuscular injections of amikacin (daily except during week-ends) at doses of 450 mg/kg. Responses were monitored every 2 to 4 days before and also during the treatment, then with longer time intervals between tests after them.

For all the guinea pigs, CAP audiograms started to alter already at the end of the first week of treatment, beginning with an elevation of the high frequency thresholds (Fig. 1). By the end of the treatments, all the CAP audiograms showed the same pattern with thresholds around 50–70 dB at 0.5 kHz and progressively increasing with the frequency up to 90–100 dB at 8 kHz.

Then shortly before or after the end of the treatment, ? guinea pigs died (at 14 and 20 days).

By this time the click-evoked responses were profoundly altered in all the animals.

Cell-mediated Immunity

some mediators in man

Mediator	acting on
Chemotactic factor	Lymphocytes, Macrophages Microphages
Blastogenic factor	Lymphocytes
Resistance-producing factor	Macrophages
Interferon	Viruses
Inhibitory factor of migration	Macrophages
— — of clon induction	Lymphocytes
— — of proliferation	cells altered by antigen

(from E Macher)

Fig 13 Some mediators in cell-mediated immunity (after E. Macher)

the invader and according to the actual defense posture of the mucosa a partial or total mobilization of all mucosa defense possibilities i begun (Fig 10)

The intercellular substance consisting of mucopolysaccharides and proteins constitutes the foundation of the lamina propria This basic substance is a very variable colloidal system forming not only a mechanical barrier but also constituting a fundamental biochemical defense factor since all reactions and functions of the tissue must proceed via the basic substance—whether they be nutritive hormonal toxic metabolic or inflammatory inflammatory processes

Unspecific and specific defense mechanisms are integrated in the lamina propria—as in the secretory film—although the active factors have a different composition compared with the secretion

The unspecific local reactions include among other things the mobilization of the neutrophilic leukocytes the activation of the macrophages and the initiation of the associated phagocytosis as well as the induction of inflammation in co-operation with the mast cells The unspecific reactions however also include the activation of the complement system and the production of interferon

The specific local defense system in the mucosa is guaranteed by humoral and cellular immune reactions In our special case of microbial penetration by way of the mucosa surface, a local immune reaction normally develops, in which both humoral and cell-mediated immunological mechanisms mutually participate

The many sometimes very complicated details with which modern immunology confronts us cannot be dealt with within the scope of this paper One should however mention a few immunological facts in this context in order to round off the topic

The lymphatic cells play a decisive role in the defense against infection The individual lymphocytes differ by a number of immunological properties It is a well-known fact that a functional differentiation is made between two fundamentally different classes of lymphocytes the B cells and the T cells In somewhat simplified form the functional relation can be explained as follows. antigens in our case microorganisms which are recognized by the organism as being foreign result in sensitization (Fig.11) In so doing both B and T cells are activated

The activation of the B cells leads to the maturation of plasma cells (Fig 12) which in turn produce specific antibodies All cells of a clone and their offspring retain the capacity to synthesize at all times the same specific immunoglobulin directed against the antigen causing the sensitization The formation of a new antibody takes about one week If on the other hand immunoglobulins against an antigen are already available in the organism, however this latency interval is shortened to an immune response of 1–2 days In this immune response the B cells co-operate with the complement system and—similar to the T cells as well—with granulocytes macrophages and other lymphocytes It may be of interest that there are roughly 10^3 receptors for antigens on a single B cell (Mims)!

T cells (Fig 12) constantly migrate through the tissue and thus through the mucosa as well While on patrol they may encounter an invading antigen in this case microorganisms. This contac

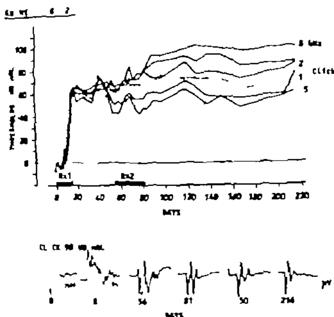


Fig. 3. Upper curves. Evolution of the thresholds to the various filtered clicks (from 0.5 to 8 kHz) and to the click ( ) during the entire experiment including the aminoglycoside treatments (Rx1 and Rx2). Note the relatively good low frequency thresholds. *Lower trace.* Click-evoked responses from the same guinea pig at various times. Traces as in Fig. 2. Peak-to-peak amplitudes indicated in microvolts in small characters. Note the disappearance of the early positive peaks during the second treatment (*days 56 to 81*). *It is not obvious whether the final response (at days 150 or 216) is present or not as the responses at 0, 18 or 56 days.*

the ears and from the auditory cortices (with contralateral acoustic stimulation) in GPs 124 and 117 (Figs. 4 and 5) while no response was *recordable either from ears or from the* auditory cortices of GP 123.

In GP 189 responses were remarkably constant both in pattern and amplitude from day 85 (implantation) to day 347 (sacrifice) (Fig. 6). Similar responses could be recorded in GP 196 (Fig. 7) while no response could be recorded from either the left and right ear of GP 195. However for this GP it was obvious either *during electrode implantation for acute recordings* or later when preparing the ears for histology that it had been suffering from bilateral otitis media (thickening of bone and presence of membranous material in the middle ears).

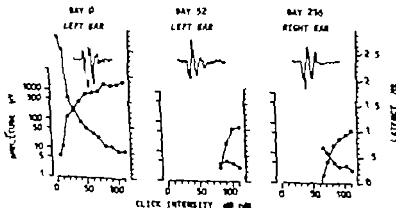


Fig. 4. Patterns of the click evoked responses at 90 dB nRL (*upper trace*) and input-output amplitude and latency functions at days 0 and 52 from the left ear and at day 216 from the right ear of GP 117 which received the same treatment (Fig. 3) as GPs 123 and 124. Traces as in Fig. 2.

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Prof Dr med H H Naumann
Direktor der Univ -HNO-Klinik
Klinikum Großhadern
Marchioninistraße 15
8000 München 70
FRG

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J.-M. Aran
Laboratoire d'Audiologie Expérimentale
(E.R.A. Inserm no. 39)
Université Bordeaux II
Hôpital Pellegrin
Place Amélie-Raba-Léon
33076-Bordeaux Cedex
France

DISCUSSION

Spoendlin to Aran. In some of your animals you recorded acoustic potentials in spite of an apparently complete loss of hair cells and spiral ganglion cells. This is in fact very surprising and can only be explained by an acoustic activation of some unaffected vestibular elements. I would also question any correlation between the ECOG response and functioning cochlear neurons. The quantitative morphological evaluation of the cochlea is of course crucial for the interpretation of these results. Such quantitative critical evaluation is probably very difficult on the basis of serial sections and I think combined surface and EM technique would be better. In our experience it can easily happen that one finds distorted rudimentary hair cells in EM preparations of cochleas where in LM all hair cells seem to have disappeared.

Barnes to Aran. It is very difficult to find an explanation for the described observation. If we assume that all hair cells are destroyed and not merely damaged and still functioning, we have to think of vestibular transformation. This vestibular source of the potentials seems to be very improbable—otherwise we would find some potentials more frequently in completely deaf patients. One hypothetical explanation seems to be a sinister phenomenon we all know named electrophony. As it is based on electromechanical transformation it could also be possible that mechanoelectrical transformation produces an electrical stimulation of the nerve fibres.

Gibson to Aran. I ask whether any adaptation studies have been performed. This would distinguish between a receptor potential or an artifactual microphonic and a truly neural component.

Brapley to Aran. This response should perhaps be called hair-cell less acoustic response rather than a cortiless response. Presumably the other cells of the organ of Corti are present?

BM Johnstone has postulated direct mechanical-stimulatory effect on the nerve fibres supplying the IHCs. It is possible that this is the origin of the response in the auditory nerve that Aran has described.

As part of the response has a short latency 0.3 ms, it suggests that some form of receptor potential is involved. It would be interesting to do d.c. recordings of the scala media to see if there is an endolymphatic d.c. potential present in the ears damaged with Amikacin.

Bauer to Aran. It would be interesting to know the frequency spectrum of the click stimulus. Assuming that it is response of the saccule, strong click may cause sudden dislocation of the stapes which might be a proper stimulus for the saccule. If this is so, have you observed any movements of the eyes by nystagmography?

Aran (Portmann repl.)

Thank you for your comment. In the absence of Dr Aran I will try to do my best and invite you to our laboratory to see exactly what Dr Aran and his group do.

They carefully observed with light and electron-microscopy and they believe that the hair cells are destroyed.

The behaviour of the response proves that it is not an artefact. I thank the speakers for the ideas they gave to try to explain the phenomenon.

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Prof Dr med H H Naumann
Direktor der Univ HNO-Klinik
Klinikum Großhadern
Marchioninistraße 15
8000 München 70
FRG

sue proved to be a less good interpositum The venous wall partly calcifies is transformed and becomes stiff A part of the fatty tissue necrotizes another part forms in some of the cases, undesirable granular tissue

Before transplanting the materials we had attempted application of a low-energy helium-neon laser in order to observe how the irradiation of the low-energy laser affects the adhesion of transplantation Unexpectedly the low-energy laser promotes the vascularization and adhesion of transplantation considerably As a result of irradiation the transplant incorporates faster and vascularization also follows earlier

We have also applied irradiation to close the perforation The low-energy laser induced rapid epithelialization of the perforation.

We have applied irradiation to close experimental tympanic membrane perforations as well The low-energy laser induced rapid epithelialization of the perforation It was shown by the histological investigation carried out after the low-energy laser irradiation that no change arose On the other hand oedema was formed at elevated energy levels

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Prof Dr O Ribari
E.N.T Clinic
University Medical School
H-6725 Szeged
Hungary

DISCUSSION

Portmann to Ribári. I think that we have to avoid gelfoam for closure of the oval w ndow I 1964 I did 300 cases with gelfoam and the sensorineural hearing loss went up from 0 7% to mor than 6%

I think that w must recommend absolute avoidance of gelfoam—if it is not toxic by itself which is not proved it does result in this membrane which does not protect the labyrinth sufficiently against inflammation from pharynx and tubal region.

Keleman to Ribári. Tissues acquire independence in late lif as in embryonic periods.

Ribári (Reply)

Thank you very much for your questions

The gelfoam made very thin membrane but it has no toxic effect.

It depends on the method of sterilization

An ideal material is fascia and perichondrium. Fat and vein are no good

Table I *Cytologic types of effusion*

Cytologic type	Serous or seromucinous effusion N	%	Mucoid effusion N	%
Granulocytic	37	65	69	58
Monocytic–lymphocytic	10	18	20	17
Mixed	3	5	11	9
Few cells	7	12	19	16
Total	57	100	119	100

Cytologic type could not be determined.

sponse and the damage caused by immune complexes possibly present in the middle ear liquid

Cellular Response

In secretory otitis media the cellular response may vary considerably There are specimens both of the serous and of the mucoid type which contain only small numbers of inflammatory white cells whereas the majority of cases show considerable inflammatory response In our material the frequency of specimens with a poor inflammatory response was 12% for the serous and sero-mucinous and 16% for the mucoid type respectively

In the microscopic examination of smears stained with Wright s or Papanicolaou s stain and cytocentrifugated cell preparations stained with May-Grünwald-Giemsa (MGG) the effusion liquids were divided into three groups a granulocytic type a monomuclear cell type and a mixed type (Palva et al 1978 1979) In the first group we included all specimens in which 60% or more of the cells were polymorphonuclear leukocytes and in the second those in which the mononuclear cells exceeded 50% The liquids in which polymorphonuclear leukocytes constituted between 50 and 60% of the cells formed the third mixed group Table I shows that most of the effusion liquids were granulocytic lending support to the view that ongoing infection is an important etiological factor Nearly 20% of the liqui[ds] were monocytic–lymphocytic

In a further subdivision of the monocyti[c–] lymphocytic specimens we used the recent[ly] discovered α naphthyl-acetate-esterase e[n]zyme (ANAE) staining method to mark th[e] T lymphocytes This histochemical stainin[g] method has been employed in studies on hu[]man specimens by Ranki et al (1976) and b[y] Ranki (1978) after Müller et al (1975) had dis[]covered that in histological sections of mous[e] lymph nodes the T-cell areas were positive t[o] this enzyme In human blood 90% of lym[]phocytes which bind sheep erythrocytes ex[]press this marker while the surface Ig-expres[]sive lymphocytes (B-cells) remain negative. A fraction of the null cells also expresses th[is] marker but as their overall number is smal[l] their effect upon the total frequency of mar[]ker positive lymphocytes is insignificant

Table II shows the lymphocyte compositio[n] as evaluated in the cytocentrifugated speci[]mens after incubation for 6 h to demonstrat[e] T-cells with the ANAE The effusion type whether serous or seromucinous or mucoid had been assessed on the basis of gross ap[]pearance and it can be seen that the per[]centage of T-cells in both liquids was compar[]able to that found in the blood i e 60% I[t] should be borne in mind however that irre[]spective of the duration of the disease 10–15% of specimens from chronic effusions had very sparse cellular populations Furthermore

Table II *ANAE positive lymphocytes in effusion*

Effusion type	Frequency of ANAE positive lymphocytes (no of specimens and percentage) 50–100 % n (%)	50 % n (%)	Total no of specimens
Serous or seromucinous	31 (68 %)	9 (41 %)	40
Mucoid	48 (64 %)	12 (35 %)	60

The figures in parentheses are the mean percentages of ANAE-positive cells.

vertically uniform There was a decrease in the density of AChE staining within the subplate layer (Fig. 4)

In oldest fetuses examined (28 weeks of gestation) a pattern of vertical darkly stained alternating zones (140–200 μm wide) appeared within the AChE-positive middle third of the cortical plate (Fig. 6 arrows) These vertical positive zones (columns) are separated by narrower interspaces of lower staining density AchE-positive columnar zones could be followed in serial sections in a rostrocaudal direction

DISCUSSION

The observation presented in this paper provides evidence that there is a characteristic distribution and evolution of AChE staining within cytoarchitectonic compartments of the developing human auditory cortex The early bilaminar pattern of AChE staining (marginal zone and subplate layer) corresponds to the bilaminar distribution of synapses (Molliver Kostović & Van der Loos 1973 Kostović Knežević, Kostović Krmpotić Nemanić Kelović & Vuković, 1978) observed in human fetal cortex Thus AChE-positive fibres may be involved in early fetal synaptogenesis The onset of AChE staining within the cortical plate at 24 weeks of gestation corresponds to the appearance of synapses in this layer (Molliver Kostović & Van der Loos 1973 Kostović & Molliver 1974) and may indicate *the establishment of thalamocortical circuitry* In accordance with this hypothesis is our finding of AChE-rich, vertically oriented alternating zones in prospective layer IV of the auditory cortex at 28 weeks of gestation This columnar pattern of AChE staining which has been also observed by us in frontal cortex of human fetus (Kostović 1979) may correspond to the columnar distribution of thalamocortical fibres found in primate neocortex (Hubel & Wiesel 1969 Rakic 1976) or granule cells in primate auditory cortex (Smith & Moskowitz, 1979) while AChE-poor zones may correspond to the termination of cortico-cortical fibres observed in the frontal cortex by Goldman & Nauta (1977) Vertically oriented AChE-positive bands may be also related to the columnar cortical organization identified by both physiological and neuroanatomical approaches (Mountcastle 1957 Hubel & Wiesel 1977 Hubel Wiesel & Stryker 1978) Thus our observations (Kostović 1979 this study) may constitute the first evidence of columnar organization in the human cerebral cortex.

As suggested previously (Vaughan 1975 Krmpotić Nemanić, Kostović Nemanić & Kelović, 1979) the early establishment of the main organization of the auditory cortex may be of essential significance for both normal and abnormal development of the central auditory system

At present we have no evidence that the AChE-positive reaction found in our material may reflect the cholinergic nature of the thalamic input to auditory cortex as was proposed for other sensory cortices (Kristt 1979) It is possible that the presence of AChE is related to the process of growth and differentiation and may be present in some developing telencephalic fibre systems (Kostović Knežević, Kostović Krmpotić Nemanić & Kelović 1979 Kostović Kelović Kračun & Krmpotić Nemanić 1979) Furthermore the AChE positive reaction may be related to some noncholinergic inputs as suggested by Emson & Lindvall (1979)

RÉSUMÉ

Dans cette étude les méthodes de coloration avec acetyl-cholinesterase (AChE) étaient adoperées pour demontrer le development de fibres afférientes du future cortex auditif dans les fetus humains de 8 à 28 semaines de gestation. La première AChE positive reaction apparait chez les fetuses de 20–4 semaines dans le neuropil de la zone marginale et dans le subplate layer du cortex auditif Par cette coloration charactéristique avec AChE le corte auditif peut étr delimité des autres regions corticales. A 4–26 semaines pendant la lamination laterauve de la zone corticale AChE-positiv reaction fut trouvée dans la partie perfonde d la zone corticale Dans les stades de 28 semaines une structure en colonnades erticale et sombre dans le tiers intermediaire de la zone corticale fut observée On peut en conclure que les fibres

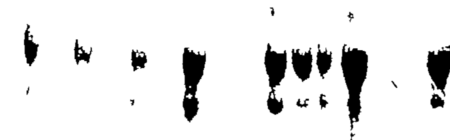


Fig 2 Similar analysis as in Fi but a more porous gel is used. T broad albumin band has shifted towards the anode Serum refe ces are on the left and right.

no protein bands that in this analysis would have proved different from those present in serum

Immune Complex Disease

Another line of research which is only now starting to gain importance is based on the proposition of Veltri & Sprinkle (1976) that the presence of a specific antibody a source of antigen and lysosomal enzymes could provide the basis for chronic immune complex damage to the middle ear mucosa. Mravec et al (1978) tested this in an animal model injecting immune complexes into chinchillas ears and found a typical complement mediated acute inflammatory reaction Recently Leinonen (1979) provided evidence that pneumococcal capsular antigens are almost invariably present (88%) in the middle ear liquids from which pneumococci have been cultured In addition these antigens were present in 33% of the no-specific pathogen liquids in which the cultured agent was *Hemophilus influenzae* In some liquids the lack of antigens was apparently due to the fact that the antige were already fixed in immune complexes deed they could be separated by heating pneumococcal antigen becoming demo strable

COMMENT

Presently two new aspects of immunologic mechanism apparently pertaining to the chr nicity of secretory otitis media seem emerge One is the normal frequency of T cells in both the serous and mucoid cell-ric effusions which as such is evidence of an ade quate T-cell response However these cell are associated with the development of de layed type hypersensitivity reactions whic arise from local contact with suitable antigens Two main antigen groups may here be of im portance viz food antigens with which the middle ear mucosa comes into contact during the bottle feeding period and bacterial antigens which attack the mucosa at any age The importance of this mechanism is still a matter for future research

Tabelle II ³H Thymidin Markierungsindex und die histopathologischen Angaben der Larynx Karzinome 1974–1978

	Differenzierungsgrad		
	Hochdifferenziert	Mittelmässig (moderately) diff	Gering. differenziert und undifferenzierte
Casus	29	31	18
³H Markierungsindex, L.I %	(5–7,8) 6,5	(7.. 10,5) 8,5	(9,8–15 1) *11,9*
Mitoseindex, M.I %	(0 7–1,3) *1,06*	(0,9–1 1) *1 43*	(1,5–..4) *1 91*
Zellkern-Morfologie (nuclear grading)	NG 3	NG 3–NG	NG 1
Necrosis	0 +	+	+
Invasion (an der Randzone)	Umgrenzend	Gering-tief (irregular)	Tief infiltrativ (highly)

Es sei betont, dass der M.I. inkubierten Gewebes immer geringer als in vivo-Verhältnissen ist, weil die im Gang befindlichen Mitosen während der Inkubation ablaufen aber neue Zellen aus der G_2-Phase in die M-Phase nicht übertreten (Rajewsky, Oehlert).

ERGEBNISSE

Auf Grund des histologischen Differenzierungsgrades sind die Kehlkopfpatienten in 3 Gruppen eingeteilt worden

- in der I. Gruppe: Kranken mit hochdifferenzierten,
- in der II. Gruppe: Kranken mit mittelmässig differenzierten
- in der III. Gruppe: Kranken mit gering differenzierten Karzinomen.

Dem Reifegrad entsprechend wurden auch die Zell- und populationskinetischen Parameter gruppiert.

Die Tabelle I zeigt die mit in vivo und in vitro ³H[TdR] Markierung bestimmten und geschätzten Werte.

Die Werte der Markierungsindices sind innerhalb einer Gruppe auch nicht gleich, trotzdem kongruieren sie mit dem Differenzierungsgrad des Tumorgewebes gut.

Die Wachstumfraktion (G.F.) erweist sich als eine andere charakteristische Angabe. Mit Abnahme der Ausreifung der Tumorzellen steigt die Zahl der proliferierenden Zellen des Gewebes. Es entspricht der von Stein abgefassten Grundregel: mit der Abnahme der strukturellen Differenzierung nimmt die funktionelle Kapazität ab und es steigt die reproduktive proliferative Kapazität des Gewebes.

Der Zellverlust beträgt mehr als 90%, stammt aus Zelltod. Migration und die Exfoliation spielt bei den Larynxtumoren eine wesentliche Rolle.

Die nach in vitro ³H[TdR] Incorporation bestimmten Angaben von 78 L.I. wurden nach dem strukturellen *Differenzierungsgrad* gruppiert. Die Ergebnisse sind in der II. Tabelle dargestellt.

Die Werte der Markierungsindices sind mit dem histopathologischen Befunden in gutem Einklang und ändern sich *zwischen* 5–15%. Ein L.I. der unter 8% liegt, ist für die gut *differenzierten* Geschwülsten charakteristisch. Der L.I. wurde nur dann über 10% gefunden, wenn das Karzinom geringdifferenziert oder undifferenziert war.

Mit den anderen morphologischen Kennzeichen der Malignität des Tumors zeigte der L.I. eine gute Korrelation.

Die Zellkernatypie ist bei den hochdifferenzierten Geschwülsten nach Bloom und Black *(NG3)* geringer, aber mit der Abnahme des strukturellen Reifegrades können sowohl in Form, als auch in der Grösse und Färbung bedeutende Unterschiede gefunden werden (Pleomorphismus: NG² NG1).

Die Ausdehnung der Nekrose bei nicht

Acta Otolaryngol 89· 182–186 1980

PROBLEMS CAUSED BY IMMUNODEFICIENCIES

J Jákó

From the Department of Medicine Postgraduate Medical School Budapest Hungary

The identification and functional classification of the various immunodeficiencies do not seem to provide the final answer to the problem From the aspect of didactics as to its tasks and function the immunoapparatus can be divided into three major areas the afferent branch the central portion and the efferent branch

I shall treat my subject according to this division

The afferent branch which takes care of antigen uptake is split into two phases one aspecific and one specific The aspecific phase includes the activity of the segmented leukocytes Should it deviate from the normal the patient as in any other deficiency of the immunoapparatus or of other areas will suffer from a persistent sino-broncho-pulmonal infection But for the clinician the picture is by no means unambiguous He recognizes the deficiency of resistance but the clarification of the basic pathological process calls for great efforts and considerable instrumentation The deficiencies of the leukocytes are mostly genetically determined

Whether the organic reaction to antigen stimulus is cellular or humoral depends on the specific phase

The first possibility is that the number of epitopes in the antigen is very small and if they attach to the cell surface there will be no more free epitopes and no interaction between the T and B cells The result will be a cellular immunoresponse

In the second consideration is the number of epitopes in the antigen greater A cell-to-cell interaction will take place since one of the epitopes attaches to the T and the other to the B cell producing a humoral antibody response

The third possibility is the presence of an antigen with a large number of epitopes in a low concentration which considering the probability of their linking is incapable of linking T and B The outcome once again is a cellular immunoresponse the phenomenon of low zone tolerance

The fourth possibility is the opposite i e an antigen with an excess of epitopes in the organism Both T and B may individually attach to the cell surface without causing cellular interaction The result once more is a cellular interaction—the high zone tolerance

The fifth possibility proved in practice is the case in which the antigen entering the organism can immediately link with a preformed

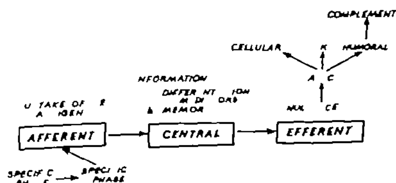


Fig 1

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Dr J Élő
H Budapest
P.O Box 112
Hungary

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J Jákó

From the Department of Medicine Postgraduate Medical School Budapest Hungary

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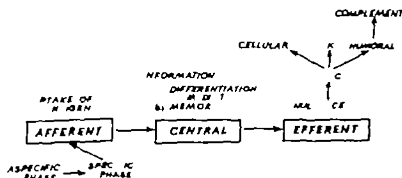


Fig 1

Acta Otolaryngol 89 401–406 1980

STREPTOMYCIN INDUCED DEFECTS OF THE OTOCONIAL MEMBRANE

Lars-Göran Johnsson Charles G Wright Robert E. Preston and Pamela J Henry

From the Kresge Hearing Research Institute Department of Otorhinolaryngology University of Michigan Medical School Ann Arbor Michigan and from the Callier Center for Communication Disorders The University of Texas at Dallas Dallas Texas USA

(Received March 23 1979)

Abstract Damage to the neuro-epithelium caused by the vestibulotoxic drugs has been well described in previous reports, however little is known about the effect of such drugs on the otoconia. In this investigation streptomycin sulfate was found to create a circumscribed defect in the crystalline layer of the otoconial membrane in the utricle of the guinea pig The defect was secondary to a sharply outlined lesion of the neuroepithelium which corresponded in size and location to the otoconial defect. Many of the otoconia along the margin of the defect showed signs of degeneration and appeared similar to those observed in the saccule of man during aging. In advanced stages of decay the otoconia were reduced to hollow shells consisting of longitudinal interconnecting strands All the epithelial lesions occurred with remarkable consistency in the same region of the macula utriculi at the posterior end of the striola.

The aminoglycosides streptomycin gentamicin, and tobramycin are the most important vestibulotoxic drugs Their major site of action is the neuro-epithelium and the damage they cause has been well documented (Hawkins 1976) This report deals with injury to the utricular otoconia caused by streptomycin.

After intratympanic application of streptomycin, the sensory epithelium of the macula utriculi shows less damage than the ampullary cristae and the macula sacculi still less (Lindeman 1969) It is of interest to note that the Type I hair cells located within the striola (Werner 1940) are more vulnerable than the Type II cells. Streptomycin is also known to affect the so-called dark cells in the membranous wall of the utricle (Hawkins & Preston, 1973)

Reports of otoconial abnormalities have been few We have observed what probably ... otoconia in cats after gentamicin (Hawkins et al 1969) and neomycin (Johnsson & Hawkins 1972) treatment. Neomycin appeared to cause loss of otoconia on the posterior tip of the saccule while gentamicin created more generalized loss of saccular otoconia with some giant otoconia remaining Because the specimens had been stored for several months in 70% alcohol the pH of which was not measured the validity of these observations is open to question. Lim (1973) has observed abnormal otoconia in the guinea pig which he believes were altered due to ethacrynic acid treatment Recently loss of both saccular and utricular otoconia has been observed in guinea pigs after administration of streptomycin (Harada & Sugimoto 1977) As has been mentioned in our preceding report experimentally induced otoconial abnormalities in guinea pigs have to be viewed in light of the fact that defective otoconial membranes do occur in untreated control animals (Johnsson et al 1979)

This report deals with an otoconial defect which with certainty can be related to degenerative changes in the neuro-epithelium The observation was made during dissection of the vestibular system in guinea pigs intended for use in a study of the effect of streptomycin on the incorportion of ⁴⁵Ca in the otoconial membranes

METHODS

The technique of microdissection which was employed in the previous study (Johnsson et al 1979) was also used in this investigation

Acta Otolaryngol 89· 182–186 1980

PROBLEMS CAUSED BY IMMUNODEFICIENCIES

J Jákó

From the Department of Medicine Postgraduate Medical School Budapest Hungary

The identification and functional classification of the various immunodeficiencies do not seem to provide the final answer to the problem From the aspect of didactics as to its tasks and function the immunoapparatus can be divided into three major areas the afferent branch the central portion and the efferent branch

I shall treat my subject according to this division

The afferent branch which takes care of antigen uptake is split into two phases one aspecific and one specific The aspecific phase includes the activity of the segmented leukocytes Should it deviate from the normal the patient as in any other deficiency of the immunoapparatus or of other areas will suffer from a persistent sino-broncho-pulmonal infection But for the clinician the picture is by no means unambiguous He recognizes the deficiency of resistance but the clarification of the basic pathological process calls for great efforts and considerable instrumentation The deficiencies of the leukocytes are mostly genetically determined

Whether the organic reaction to antigen stimulus is cellular or humoral depends on the specific phase

The first possibility is that the number of epitopes in the antigen is very small and if they attach to the cell surface there will be no more free epitopes and no interaction between the T and B cells The result will be a cellular immunoresponse

In the second consideration is the number of epitopes in the antigen greater A cell-to-cell interaction will take place since one of the epitopes attaches to the T and the other to the B cell producing a humoral antibody response

The third possibility is the presence of an antigen with a large number of epitopes in a low concentration which considering the probability of their linking is incapable of linking T and B The outcome once again is a cellular immunoresponse the phenomenon of low zone tolerance

The fourth possibility is the opposite i e. an antigen with an excess of epitopes in the organism Both T and B may individually attach to the cell surface without causing cellular interaction The result once more is a cellular interaction—the high-zone tolerance

The fifth possibility proved in practice is the case in which the antigen entering the organism can immediately link with a preformed

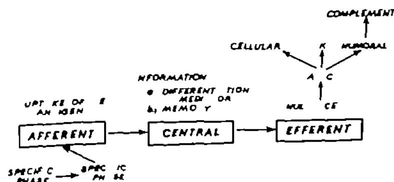


Fig 1

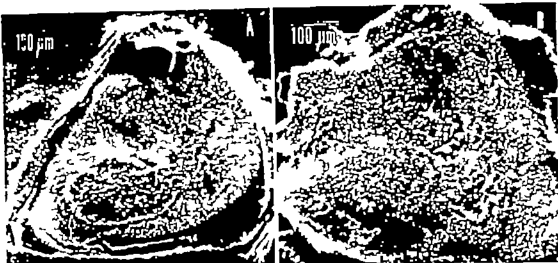


Fig 3. Scanning electron micrographs of the right utricular maculae from 2 animals killed 20 days after treatment. The tufts of sensory cells are displayed. The posterior tip of the macula is in the lower left and right corners respectively. *A*, Single large lesion. The margin of the defect is thickened (*vertical arrow*). *B* T small defect (*horizontal arrow*). Not the sensory cell los along the striola.

clearly accentuated the original defect in the otoconial membrane

DISCUSSION

This study has demonstrated a more or less precise local correlation between a defect in the crystalline layer of the otoconial membrane and a streptomycin-induced lesion in the neuro-epithelium of the macula utriculi in guinea pigs. One can only speculate about the process which caused the loss of otoconia. It is highly improbable that purely mechanical factors were involved. We believe it is more likely that the epithelial damage interfered in some way with the calcium metabolism of the otoconia. Recent studies (Preston et al. 1975, Mechigian et al. 1979) have provided evidence for active turnover of calcium in the otoconial membranes. Thus the streptomycin treatment may have disturbed biochemical mechanisms responsible for calcium transport or uptake. Also during degeneration of the neuro-epithelium, lysis of cells probably occurred leading to the release of lysosomal products and enzymes which may have lowered the pH sufficiently to cause dissolution of the otoconia. Such products of cellular degeneration might in addition alter the organic matrix of the otoconia as well as the gelatinous portion of the otoconial membrane.

Our findings show little similarity to the otoconial changes recently attributed to streptomycin treatment by Harada & Sugimoto (1977). The degenerating otoconia in our specimens did appear similar to those found in the human saccule during aging (Ross et al. 1976) which suggests that a common process might be involved in production of the two seemingly different forms of otoconial degeneration.

This study does not show whether loss of sensory cells and supporting elements alone unaccompanied by focal defects of the maculae can alter the otoconia. It seems likely that this will occur with time (Mechigian et al. 1979).

The marked loss of Type I sensory cells in the utricle was to be expected (Lindeman 1969) but the circumscribed lesion occurring consistently at the posterior end of the striola was not anticipated. This finding suggests the presence in the utricle of a *locus minoris resis*

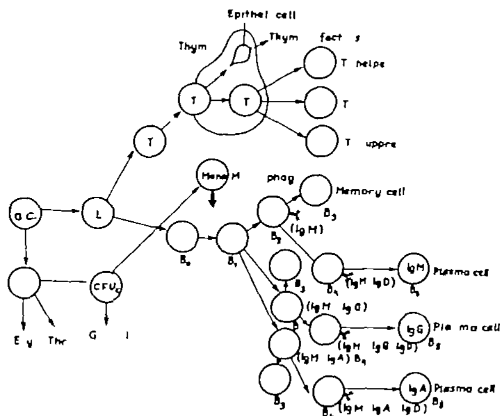


Fig 4 According to ... partly modified by th... thor

of the T and B systems in the form of the Di George or the Bruton type immunodeficiency

Between the two extremes however there are numerous transitional states which to a certain degree bear proof of the deficiency of the T–B system prevailing simultaneously or separately

The lowered resistance of the aged as an indication of the senescence of the immuno-apparatus finds partial explanation in the anomalies of the central portion

According to present knowledge the n... role is played here by the transfer factor the interferon as the transmitter sensing stimulating the process—although other called lymphocyte factors also have a sha... it

The efferent branch is responsible for fence It should be noted however that defensive system of the organism is a c... defence and not an apparatus to suppress ternal infections Every protein whose st...

SPECIFIC SYNDROMES	CELLULAR MMUNODE FICIENCY (W TH A RELA TIV SUFF CIENCY OF HUMORAL SYSTEM)				BOTH CE ULAR AND HUMORA S S EMS ARE INVO ED				HUMORA MMU NODEFICIENCY (WITH A RELATIVE SUFFIC ENCY OF CELLULAR SYSTEM)		
	DI GEORGE	NEZELOF	WISKOTT ALDRICH	ATAXIA TELEANGIECTATICA	THYMOMA ACCOMPANIED BY IMMUNODEFICIENCY	SELECTI E DISTURBANCE OF THE PRODUC ION OF IMMUNOGLOBULINS (IgA DEFICIENCY)	GROUP OF DYS γ GLOBULINEMIA (DE IN ED ON WITH D FICU TY)	SW SS TY E OF AGAMMA GLOBUL NEM A	PRIMARY CONGEN TAL AGAMMAGLOBU NEM A	TRANSITORY H POGAMMA GLOBULINEMIA IN CHILDHOOD	BRUTON

Fig 5

glass in inverted-tip type electrodes Because a 1 mV error in the reading is only equivalent to a Na concentration change of 0 04 mM at the endolymph concentration spatial separation of the tips of the electrodes was not a serious problem In a subsidiary series of experiments, single pH microelectrodes of the recessed-tip type (tip diameter ?.5 μm) made from H -sensitive glass (Corning 0150) were used in place of the Na -sensitive electrodes

Repeated measurements of the calibrating solutions always gave identical readings and a better estimate of the sensitivity of the methods was therefore required. All potentials were recorded to ±50 μV For the Na sensitive microelectrode this was equivalent to ±0.002 mM at the 1 mM level and ±0 0? mM at the 10 mM level The corresponding figures for K were ±0 28 mM at 150 mM and for pH ±0 001 unit at pH 7 4 The results are routinely expressed in the form mean ±standard error of mean number of experiments (unless this is given in the adjacent text) The values give ionic concentrations and not activities to enable comparison with other findings to be made more easily

Permeability and flux calculations

Due to the absence of a suitable direct method permeability alterations were quantified by means of the principle introduced by Hodgkin & Katz (1949) and developed for the cochlea by Johnstone and his colleagues (Johnstone 1970 Sellick & Bock 1974 Sellick & Johnstone 1975) In essence the rate of change in the endolymphatic concentration of the ion concerned for any selected moment during terminal anoxia, normalised with respect to the relevant electrochemical gradient is used to calculate the conductance of the ion at that moment according to the equation

$$\lambda G = \frac{\Delta I}{\frac{RT}{zF}\ln\frac{a^l}{a^l} + \Delta E}$$

where R is the gas constant, T is the absolute temperature is the valency of the ion F is Faraday s constant a^l and a^l are the activities of the ion in endolymph and perilymph respectively ΔE is the electrical potential difference between endolymph and perilymph ΔI is the rate of change in the endolymphatic concentration of the ion G is the true conductance and λ is a constant Although λ is specific for any particular animal species its value is not known precisely and the term λG_i is taken as an index of the permeability of the ion which enables relative permeabilities and permeability variations to be determined accurately In anoxia its value remains unchanged for 3–6 min after the minimum peak in the endocochlear potential following which substantial increases in cation permeability occur The cationic conductances during this short stable period have been shown to be representative of the pre anoxic conditions (Bosher 1979)

When multiplied by the appropriate ionic driving force conductances determined in this way provide an estimate of the rate of concentration change due to the passive forces at any time during the experiment. Thus the contribution of the active transport processes can be derived from the net concentration change according to the equations

$$cJ_{pass} = \lambda G_i \left(RT\ln\frac{a^l}{a^l} + \frac{EP}{F}\right)$$

$$cJ_{activ} = cJ_{net} - cJ_{pass}$$

where J_{active}, J_{net} and J_{pass} are the active net and passive ionic fluxes at time t c is the volume area constant for the endolymph system of the animal (so cJ is the rate of concentration change) and EP is the endocochlear potential While the true fluxes (J) cannot be specified because c has not been measured with sufficient reliability the results do enable the various ionic fluxes to be compared with each other and the changes in the fluxes with time can be followed

DEFICIENCY OF C_5
DEFICIENCY OF C
CONGENITAL H PERCATABOLISM OF C_3
FAMILIAR DYSFUNCTION OF C

Fig 9

All congenital anomalies caused by lack or dysfunction of the individual complement factors will manifest themselves in a series of infections

Summing up In the time allocated and due to the large volume of information it was impossible to go into greater detail on this extremely interesting subject I have attempted to give a survey of the functioning of the immunoapparatus from the aspect of the antibody deficiency syndromes However I would like to call attention to the fact—which follows unambiguously from this lecture—that if the case in hand is suspected of immunodeficiency not only must the immunoapparatus be examined but very often also the macrophages the leukocytes and the complement system Fortunately genuine immunodeficiencies are few and far between but the clinician is often confronted with pathological processes which differ from the commonplace Some transitions may exist which are not immunodeficiencies merely a weakness of the immunoapparatus and should this happen the physician may further aggravate the situation for instance by antibiotic therapy

RÉSUMÉ

La brièveté de temps et la multitude des données diversifiés ne m ont pas permis de m approfond dans les détails
extrêmement interessantes Le but que j'ai pou...
embrasser d'un coup d'œil la fonction de l'appareil im
nologique sous l'angle des symptomes défectueux des
ticorps. J'attire l'attention ce que d'ailleurs ressort de
tement de mon cours a ce que si le soupçon d'une d
cience immunologique subsiste il s'impose non seule
la prospection dans le sens étroit du mot de l'app
immunologique mais souvent l'examen des glob
blancs des macrophagues et du système complément
Heureusement la vraie déficience immunologique est
maladie des plus rares mais le clinicien constate sou
un aspect pathologique différent du processus habi
Probablement ils existent des transitions ou il ne s agi
de déficiences immunologiques amis des simples fai
ses et dans ce cas d'une façon inconnue le médecin
jour comme facteur affaiblissant un certain rôle, p
par suite d'une thérapie antibiotique

ZUSAMMENFASSUNG

Die Zeit, die mir zur Verfugung stand sowie die Viel
weltverzweigter Angaben haben es ermöglicht, wo u
einzelnen außerordentlich interessanten Detailfragen
vertiefen Mein Ziel war über die Funktion des Imm
apparates vom Gesichtspunkt der Syndromen mit
mundefizienz Antikörpermangel einen Überblick zu
ben. Ich möchte die Aufmerksamkeit darauf lenken
folgt aus meinem Referat eindeutig – daß im Falle
Verdachtes auf ein Krankheitsbild von Immundefizi
nicht nur der Immunoapparat selbst sondern oft auch
Leukozyten die Makrophagen und auch das Kom
mentsystem untersucht werden müssen Glücklicherw
ist die echte Immundefizienz eine außerordentlich sel
Krankheit. Viel ofter sieht der Kliniker ein Bild, das
gewöhnlichen Krankheitsablauf abweicht Höchstw
scheinlich gibt es solche Ubergange bei denen es s
nicht um die Immundefizienz, sondern nur um e
Schwäche handelt. In diesen Fallen kann auch der Ar
auf bisher unbekannte Weise – z.B mit der Thera
durch Antibiotika als ein weiterer erschwerenden
Faktor mitwirken

J Jako M D
Dep of Medicine
Postgraduate Med School
POB 112
H 1389 Budapest
H ng ry

...er in the case of Na but faster in the case of K. As described below these findings (Fig. 7) indicate that ethacrynic acid given intravenously has essentially the same actions in the two species, the differences found being only of a minor quantitative nature.

Permeability alterations

Each experiment was terminated with anoxia induced at varying times after the initial injection and the relative alterations in the permeabilities of the endolymphatic membranes which had arisen were calculated as described earlier. The results (Fig. 3) showed that the ethacrynic acid had produced decreases of 49(±4)% in the Na permeability ($n=10$) and 78(±3)% in the K permeability ($n=9$) in the rat. These permeability effects were present at 30 min and were long-lasting, no evidence of recovery being present at 120 min. Attempts to determine the time course of the reductions more precisely were unsuccessful. This was because some ethacrynic acid probably remained in the inner ear tissues when anoxia was induced shortly after the drug's administration. In these circumstances, the permeability changes would eventually develop fully during the anoxic period due to its continued action. It will be noted that these permeability effects proved to be different in type to those expected from our preliminary investigation (Bosher et al. 1973). An advantage of the dose of ethacrynic acid selected was that the rise in the endolymphatic Na concentration was well below the level of 12 mM. This is the concentration which is associated with a marked increase in the permeability of the endolymphatic membranes to cations (Bosher 1979). The complications of a secondary change of this type were therefore completely avoided. The guinea pig experiments were too few for the total range of the effects to be determined accurately. But the mean reductions found in the Na and K permeabilities were 45% and 68% respectively (i.e. about the same as in the rat).

Effect on active transport

The changes in the endolymphatic ion concentrations provide very little information by themselves about the effect of ethacrynic acid upon the active transport mechanisms. The marked alterations in the endocochlear potential produce equally marked alterations in the passive fluxes and these must be taken into account first. However, this can be done as described earlier and the action upon the ion-transporting mechanisms is then revealed.

In every animal, the active processes responsible for Na and K transport and endocochlear potential production were completely abolished by the time of the minimum peak in the potential. A problem arises about the exact interpretation of the details of this action. As explained above, it was not possible to determine experimentally the precise time courses at the beginning of the permeability effects. So the initial time courses illustrated in Fig. 3 were used in the calculations. Although these were undoubtedly good first approximations, the early differences between the various processes apparent in Fig. 4 might have been due to small variations in the true time courses of the permeability effects rather than actual divergences between the processes themselves. For example, the initial small rise in Na activation might have been due to a slight transient increase in Na permeability before it declined to its later level (Fig. 3). Whatever their cause, these initial differences were relatively minor in nature and activation was reduced to zero at the time of the minimum endocochlear potential in every case. Consequently, the principle effect of the ethacrynic acid in this respect was to fully inhibit all the transport processes measured at about the same overall rate.

At and after the time of complete inhibition the reductions in permeability measured individually could be used in each experiment. Thus, similar problems do not arise about the findings in this period. These revealed that the Na transport increased much more quick-

cytes was investigated in repeatedly inflamed human tonsils simultaneous with the study of their morphologic structure

MATERIAL AND METHODS

Patients

20 patients (10 male and 10 female average age 19 4 and 8 9 range 3–44 and 3–24 years respectively) were tonsillectomized because of repeated tonsillitis Tonsils of 3 dogs were used for comparison

Lymphocyte activation test

Immediately after excision the tonsils were placed into ice-cold 0 9% saline soon after (30 min) a cell suspension was prepared by mechanical disaggregation of the tonsils in a culture medium (RPMI 1640 Difco) The cells were counted in a haemocytometer and 1×10^5 viable cells in 0 01 ml culture medium were dropped on glass slides dried and stained with rivanol according to Kellermayer & Jobst (1970) and a polarization optical analysis was performed (Surján & Sebők 1973) The frequency of nuclear birefringence of tonsillar lymphocytes was determined and the presence of activated cells with increased birefringence was sought in addition to the normally distributed non activated cell population The differences between the observed distribution and the normal distribution were analysed by means of the χ-square test

Light and electronmicroscopy

The tonsils were fixed in a phosphate buffered (pH 7 2) 4% formaldehyde solution embedded in paraffin cut and stained with haematoxylin and eosin In 5 cases the ultrastructure of the tonsils was also investigated For that purpose the samples were cut into small pieces (1 cubic mm) fixed in 5% glutaraldehyde in phosphate buffer postfixed in a 1% osmium solution dehydrated and embedded in Araldite Semithin sections stained with toluidine blue were prepared for selection of tissue blocks used in electromicroscopy The thin sections were stained w uranyl acetate and lead citrate

RESULTS

1 *Structure of the reticular epithelium in flamed human tonsils* The tonsillar cr epithelium was invaded by lymphcytes, l phoblasts plasma cells macrophages granulocytes These cells arrived at the superficial cells of the epithelium via in epithelial passages While in healthy anim the epithelial barrier between crypt lumen lymphoid cells consists of a single layer o cells characterized by abundant intrac plasmic tubulo-vesicular system (Fig. 3) in flamed human tonsils the surface of retic epithelium is covered by flat strati epithelial cells and the M cells are found o occasionally (Fig 1) The intra-epithelial p sages are usually covered with squam epithelium (Fig 2) The M cells if pres have a reduced tubulo-vesicular transport s tem (Fig 4) At some places the sur epithelial cells contain lysosomes myelin ures or rough surface endoplasmic reticul

2 *Nuclear birefringence of unstimul lymphocytes from human tonsils removed cause of recurrent inflammation* The m retardation and standard deviation of b fringent human tonsillar lymphocyte nu (18 37±4 3 nm) are compatible with those the animal tonsillar lymphocytes (16 33± nm) (Surján 1977) The birefringent lymp cytes were however normally distributed all but 3 cases (χ-square test) The probabi of occurrence of all cells having retardat equal to or greater than the mean +1.5 S D

Fig 1 Semithin section from a repeatedly inflamed man tonsil Only one follicle-associated M cell in the c epithelium ×100

Fig 2 Structure of metaplastic stratified squam epithelium from repeatedly inflamed human to ×4 000

Fig 3 Detail of a canine tonsillar M cell Abundant icles are present in the endoplasm 1 000

Fig 4 M cell in repeatedly inflamed human to There are only few cytoplasmic vesicles ×1 000

1979) However the location and extent of the biophysical component of the endolymphatic membranes responsible for the overall selective permeability has yet to be determined A possible third component of the endocochlear potential has been described in the guinea pig (Sellick & Johnstone 1974 Kusakari & Thalmann 1976 Kusakari et al 1978) but has been shown to be completely absent in the rat (Bosher 1979) Consequently it does not complicate the interpretation of the experiments on this species reported here

In the 9 rat experiments in which simultaneous Na and K results were obtained the mean diffusion potential calculated from the standard Hodgkin & Katz (1949) equation and the measured values at the time of the minimum potential peak was $-20.0(\pm 4.4)$ mV The average endocochlear potential actually found was $-24.7(\pm 3.9)$ mV the discrepancy of $4.7(\pm 0.9)$ mV being identical with the one described in other situations (Bosher 1979) The difference in the value of this negative peak to the one found after anoxia in normal animals is due to the decrease in the relative permeability of the endolymph system to K after ethacrynic acid the measured Na K permeability ratio at this time being $0.63(\pm 0.08)$ compared with the normal 0.27 in the rat

Finally the results also provide evidence about the relationship between the mechanisms responsible for cation transport As reported, the normal ratio of active K to Na transport is 13 1 in the rat and 8 1 in the guinea pig. Although the latter value is less than the 66 1 derived theoretically for the guinea pig by Sellick & Johnstone (1975) it is still well outside the coupling ratios of the Na K activated ATPases In addition the large differences in the active transport systems demonstrated during the recovery period indicate that most of the K transport must be independent of the Na transport. These findings consequently provide further experimental evidence in support of the view that some enzyme other than a Na K -activated ATPase is directly responsible for the potential generation and at least most of the K transport (Kuijpers & Bonting 1970*b* Sellick & Bock 1974 Sellick & Johnstone 1975 Paloheimo & Thalmann 1977 Kusakari et al 1978) It follows from this that the K transport will be largely and possibly completely electrogenic in nature and this is confirmed by the close association between the K transporting and potential-producing systems described in the recovery period

The importance of Na K -activated ATPase in the endolymph system has not been in question since the initial studies of the effects of ouabain (Konishi & Mendelsohn 1970; Kuijpers & Bonting 1970*a*) Moreover it is present in the stria vascularis in a high concentration (Kuijpers 1974 Kuijpers & Bonting, 1969 Matschinsky & Thalmann 1970) but it cannot be concerned directly with endolymphatic cation transport. Instead preliminary results in this laboratory (Bosher unpublished observations) suggest its role to be an indirect one such as maintaining the internal ionic environment of the transporting cells

ACKNOWLEDGEMENT

I am grateful to the Department of Medical Illustration The Middlesex Hospital for preparing the diagrams

ZUSAMMENFASSUNG

Endolymphatische Veränderungen infolge intravenöser Injektion von Ethacrynsäure an Ratten, 60 mg kg^{-1} wurden bis zu 120 Minuten lang beobachtet unter Verwendung konventioneller und ionensensitiver (Na K und pH) Mikroelektroden Man fand als Ursache 3 deutliche Wirkungen auf das Endolymphsystem. Zunächst wurden die strialen potential-produzierenden und kationen-transportierenden Prozesse total gestoppt. Wiederherstellung begann schnell und war zunächst rapide. Dann verlangsamte sie sich erheblich, vermutlich infolge der Abnahme der strialen Energieproduktion mit langsamem Beginn und langer Dauer Gleichzeitig mit diesen Wirkungen auf die aktive Transportation trat eine Veränderung der gesamten Kationenpermeabilität des Endolymphsystems auf Dies hatte einen verschiedenartigen Zeitablauf und betraf K erheblich mehr als Na Die Befunde gaben außerdem weitere Informationen über den Mechanismus, der für die normale Beschaffenheit

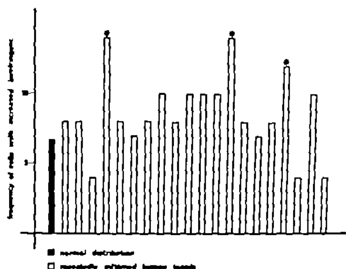


Fig 5 Frequency of the cells having equal or greater nuclear birefringence than the mean +1.5 S D Statistically significant differences from the normal distribution were found only in 3 cases (*asterisks*)

6 68% under conditions of normal distribution The occurrence of such cells in the 20 human tonsils is demonstrated in Fig 5 In 3 cases these cells with high nuclear birefringence were present in 14 12 and 14% and the difference from the normal distribution is statistically significant with a probability of 0 001 0 05 and 0 01 respectively

3 *Histology of tonsils with and without lymphocytes of increased nuclear birefringence* Histology of the three tonsils with increased nuclear birefringence (patient age 3 3 and 6 years respectively) was contrasted with that of tonsils containing normally distributed birefringent lymphocytes Tonsils containing activated lymphocytes have a faucial surface epithelium which is not (or is only slightly) infiltrated with lymphoid cells The subepithelial conjective tissue is also almost free from lymphoid infiltration (Fig 6) By contrast the surface epithelium of the other tonsils is infiltrated by lymphocytes and thus this epithelium reminds one of crypt epithelium (Fig 7) The crypt epithelium of the three activated tonsils is highly reticular and the epithelial barrier between crypt lumen and lymphocytes is narrow usually consisting of only one cell layer (Fig. 8) The crypts of the other tonsils are lined by a stratified and i some places keratinized epithelium (Fig 9) and M cells are seen only exceptionally Fibrosis of varying degrees was observed in the inflamed tonsils even in young children The lymphocyte mantle of follicles usually reached the reticular epithelium but in some inflamed tonsils a wide extrafollicular lymphoid tissue is interposed (Fig 1)

DISCUSSION

The lack of appropriate controls is the m difficulty in the study of human tonsils Animal tonsils are useful merely for comparison purposes The value of autopsy material is questionable since child mortality is happily low and adequate control tonsils are hardly attainable Furthermore the disease of the patient might affect the tonsillar structure and/or function The use of tonsils obtained from forensic dissections may be an alternative solution of the problem but the post mortem changes al so raise difficulties for the investigation of structural or functional details The best is the study of tonsillar biopsies from healthy volunteers but this has certain limitations too the small volume of biopsy material is perhaps not perfectly representative of the whole tonsil.

While researchers without appropriate controls are unable to develop diagnostic method for tonsillar diseases and there is a continuing debate over the effectiveness of tonsillectomy this operation is the most common surgical procedure It is almost certain that not only diseased but also healthy tonsils are ectomized with unknown frequency There are at presen

Fig 6 Faucial surface epithelium of a human tonsil ha activated lymphocytes ×700

Fig 7 Faucial surface epithelium infiltrated by lymphocells from a repeatedly inflamed human tonsil ×700

Fig 8 Reticular crypt epithelium from a human tons containing activated lymphocytes. ×300

Fig 9 Crypt epithelium from a repeatedly inflamed human tonsil There is stratified non-reticulated squamous epithelium on the surface ×300.

Acta Otolaryngol 89 419–423 1980

SPEECH DISCRIMINATION OF PATIENTS WITH HIGH FREQUENCY HEARING LOSS

H Klukaanniemi

From the Department of Otolaryngology University of Oulu Oulu Finland

(Received March 7 1979)

ract Mean speech discrimination curves were measd in three groups of patients having severe moderate mild high-tone hearing losses in pure-tone audioms, 20 patients being tested in each group The audioms sloped linearly by 22 dB/oct. Recruiting ears in Re test were excluded. The group with moderate hear loss achieved significantly poorer discrimination res at high sound pressure levels than the group with re hearing loss. phenomenon not reported before ever the results agree with preceding work in which valent sensorineural hearing losses were simulated h filter equipment The causes of the phenomenon and significance for hearing aid selection are discussed.

ical experience has often given the impresn that some patients with relative serious -pass hearing loss can utilize the remnants their hearing better than some with moder but in other respects similar hearing loss an earlier experiment made to examine this enomenon, three degrees of low-pass hear g loss were simulated using a filter apparatus d the corresponding discrimination curves easured using young subjects with normal aring (Klukaanniemi *1979a*) It was shown at theoretically there really are some sigficantly disadvantageous high frequency aring losses which may yield markedly orer discrimination scores at high sound essure levels than other more serious bear g losses of the same type

One case of this kind proved to be a linearly creasing hearing loss of 22 dB/octave beging at about 250 Hz. The discrimination rve for this hearing loss crossed the curve elded by a parallel hearing loss beginning at 25 Hz. The crossover point was 84 dB re 2×10^{-5}N/m² and 75% discrimination. This phenomenon has not been reported before in the literature and it encouraged the author to attempt a measurement of the discrimination curves of patients having an equivalent clinical high frequency hearing loss A further indication for this study was the lack of earlier re sults concerning the discrimination ability of Finnish-speaking patients with various grades of high frequency hearing loss

MATERIAL AND METHODS

72 patients whose pure tone audiogram showed typical high frequency hearing loss were selected for further examination An anamnesis was taken in order to outline 1) etiology of the hearing loss 2) use of a hearing aid or other auxiliary facilities 3) the patient s subjective opinion on his/her difficulties in daily life

The ear status and pure tone threshold of the patients were examined and those showing recruitment in Reger's test were excluded so as to achieve a loudness function and its influence on the threshold curve equal to the pre ceding experimental study The elimination of recruiting ears may increase the relative number of retrocochlear losses in the present material The preceding experimental study with filtered words using normal-hearing subjects gave a reason to anticipate a very disadvantageous high frequency hearing loss among non-recruiting ears. Discharging ears were similarly eliminated from the subsequent tests

no worldwide accepted criteria to sort out the healthy tonsils from the diseased ones

Earlier publications suggest that histology is suitable for this kind of selection Eighty ton sils with relatively slight or without any note worthy lesions in the histopathological appear ance were selected from 165 cases of tonsil lectomy under the diagnosis of the enlarged tonsils or chronic tonsillitis by Awaya (1969) From 107 cases of clinically chronic tonsillitis 96 have shown the histologic features of chron ic tonsillitis in preoperative biopsies (Papu row Malewa & Daskalow 1972) According to the latter authors the reticulation of crypt epithelium is not normal but suggestive of inflammation However the reticulation is a physiological phenomenon in tonsillar crypts (Falk & Mootz 1973) The histologic criteria of chronic tonsillitis were never described and the value of these criteria has never been proved in comparison with normal controls Weibel (1965) did not detect any significant ab- normality except some fungal infection his pathohistological study consisted of 4 680 pa tients According to his suggestion routine hi stological examination of tonsils should be done only after the age of forty Others have found malignant diseases of tonsils which could not otherwise be diagnosed and treated in time except by systematic histologic studies (Nicoucar & Schrago 1969 Sodagar & Mohal latee 1972) Considering that histology is not suitable for the objective testing of chronic tonsillitis other methods were suggested for the characterization of clinical stages of tonsils (Sprekelsen 1975 Siegel 1978)

The results presented here suggest that a distinction of healthy tonsils from chronically inflamed ones might be possible Our material was divided into two groups one with a nor mally distributed birefringent lymphocyte po- pulation and the other containing an additional group of lymphocytes with increased nuclear birefringence Lymphocyte nuclear birefrin gence increases after phytohaemagglutinin (Surján & Sebők 1973) and antigen (Surján 1977) stimulation This increase in the bire fringence is thought to be mediated by a sol factor of lymphocytes (Surján Juhász & bők 1979)

Nuclear birefringence of lymphocytes only increases in the course of *in vitro* stim tion but it is constantly increased due pathological conditions connected with normal lymphocyte activation such as rheu toid arthritis (Sebők Talerman & Wont 1977) Another gut associated lymphoid org the Peyer s patche contains lymphocytes increased birefringence compared lymph nodes or spleen (Surján & Kasz 1978) These data suggest that the increase nuclear birefringence means activation lymphocytes hence the lack of lymphocy with increased birefringence in all but 3 flamed human tonsils suggested a redu lymphocyte activation in the course of rec rent inflammation The reality of this obser tion is supported by the histological investi tions Tonsils which contained lymphocytes increased birefringence were similar clinically normal tonsils (Brandtzaeg Surján Berdal 1978) and the other without increas nuclear birefringence were similar to the peatedly inflamed tonsils which had reduc immunocyte density (Surján Brandtzaeg Berdal 1978)

The presented observations suggest a h pothesis the metaplastic change in tonsil crypt epithelium might be of importance in t pathogenesis of recurrent tonsillar infection The repeated inflammations of tonsils pr duces a metaplastic crypt epithelium whic contains fewer antigen receptor M cells th before which has stratified squamous cells the luminal part of the epithelium and whic is in some places not reticulated at all Simil changes are well known in the adenoid where the difference of the original ciliate columnar epithelium from the metaplast stratified squamous one is easily recognise The metaplastic epithelium forms a barrier be tween the lumen and the lymphoid tissue an therefore the activation of the lymphocyte decreases and the number of Ig-containing cell

compared with that of the English speaker although there are large individual differences in speech spectra (Byrne 1977) the linguistic of the Finnish language may also add to the errors caused by upward masking of the formant F in group C. Conversely however it also appears from the present results to be easier to utilize the remnants of one s hearing if one s audiogram belongs to groups B or D. Upward masking probably effectively eliminates the advantage which some Finnish patients (group C) with high frequency hearing loss have due to the fact that the speech power in their language is concentrated in the frequency area where their residual power of hearing lies.

The present results and those of the preceding experimental study suggest that the sloping frequency area where the disadvantageous masking effect is present is rather narrow about ½ octave. Nevertheless mild high frequency hearing losses are frequently located precisely in this area. These facts make it important in clinical practice to single out patients belonging to group C when selecting a hearing aid. The distorting masking noise of the first formant is best eliminated by a hearing aid in which amplification starts at 500 Hz. As seen here the patients in this group subjectively chose such a hearing device because of its significantly better discrimination ability. Another possibility would be a device which would present the main frequency areas of F and F to opposite ears. Some patients however show a third type of masking, central masking (Danaher & Pickett, 1974) which eliminates even the advantage of this kind of hearing aid. Also the exact separation is difficult, because F and F very often overlap.

ZUSAMMENFASSUNG

Sprachverständlichkeitskurven für drei Gruppen von Patienten mit einem schweren, mittelschweren und leichten Hörverlust wurden mit den finnischen PB-Wörtern gemessen 20 Patienten wurden in jeder Gruppe untersucht. Das Audiogramm jedes Patienten stieg parallel 22 dB/Oktave. Die Ohren mit dem positiven Rekruitment in Reger' Tests wurden ausgeschlossen. Die Gruppe mit dem mittelschweren Hörverlust erreichte bedeutend schlechtere Diskriminationswerte auf besten Lautstärken als die Gruppe mit dem schweren Hörverlust – eine Erscheinung, die man nicht früher rapportiert hat. Die Resultate stimmen doch mit dem früheren Experiment überein, wo die äquivalenten sensorineuralen Hörverluste mit einem Filterapparat simuliert wurden. Die Ursachen und die Bedeutung dieser Erscheinung für die Auswahl des Hörapparates wird diskutiert.

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Dr Heikki Kiukaanniemi
Dept of Otolaryngology
SF-90220 Oulu 22 Finland

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László Surján Jr MD
Institute of Pathological Anatomy
Postgraduate Medical School
POB 11
H 1389 Budapest
Hungary



Fig. 1. The macula of the saccule following ultrasonic irradiation towards the vestibule with 100 milliwatts (H&E, 200 ×). es, Endolymphatic space; n, neuroepithelium, om, otolithic membrane.

1.5% veronal buffered osmium tetroxide (Bredberg et al. 1965). The results were compared with that occurring after a 4-week recovery period, assessed from conventional histological methods involving Heidenhain's susa fixation, nitrocellulose embedding, serial sectioning, and staining with haematoxylin and eosin.

RESULTS OF VESTIBULAR IRRADIATION

Vestibular disturbances observed immediately after recovery from the anaesthesia included paralytic nystagmus accompanied by a head bobbing motion and loss of balance control with the cat falling onto its irradiated side. The severity of these effects was dependent on the intensity and duration of irradiation with the strongest reaction following application of 100 milliwatts for 20 min. The direction of irradiation was found to be critical and was dependent on ultrasound being aimed towards the position of the ampulla of the superior semicircular canal. In most cases completely normal locomotory function was regained within ten days following the irradiation. These symptoms of balance dysfunction indicated vestibular irritation and animals not displaying this response were assumed to be incorrectly irradiated and were excluded from subsequent histological examination.

Four weeks after vestibular irradiation histological changes were observed within the vestibular neuroepithelium. Due to the variability of cellular changes it was impossible to relate the extent of damage to the ultrasound dosage and the following changes were observed following 100 milliwatts irradiation. In the saccule the otolithic membrane was often lifted away from the neuroepithelium (Fig. 1) causing damage to the hair cell cilia. The sensory epithelium lacked clarity of cellular detail and intercellular spaces occurred in this layer and in the underlying stroma. The basement membrane separating the epithelial layer from the stroma was not clearly differentiated. The otolithic membrane in the utricular macula was more resistent to damage but cellular disruption and cilia loss within the neuroepithelium was widespread. The cell walls were destroyed and the nuclei of the supporting cells and sensory cells clumped together into thin strands resulting in disorganisation of the normal stratified appearance of this epithelial layer (Fig. 2). Cellular detail in the underlying matrix was somewhat obscure.

Degenerative changes occurred to a lesser degree within the cristae in the ampullae of the semicircular canals. Deformities of the cupula commonly occurred in the superior ampulla, together with loss of cellular detail in the neuroepithelium. The extent of these changes was variable but there was a tendency for the utricular side of the crista to be more severely damaged than the semicircular canal side (Fig.

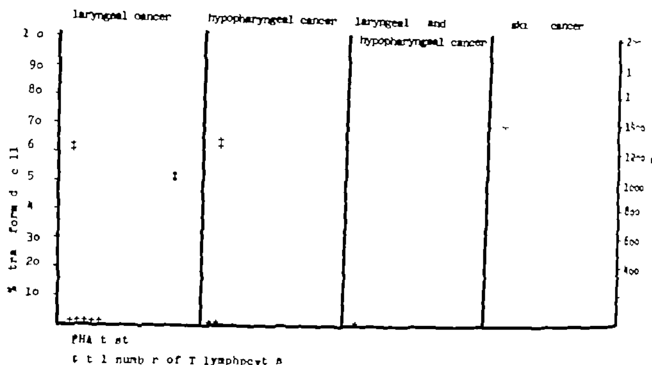


Fig 1 PHA test and total number of T-lymphocytes in laryngeal hypopharyngeal and skin cancer

genic tumors bear antigens specific for the virus they crossreact with antigens of tumors caused in different organs of the same host but also with tumors caused in different individuals

In 16 cases of laryngeal cancer we tested two tumor antigens which we obtained from squamous cell carcinoma and from corneous squamous cell carcinoma—the two most common tumors of the larynx Tumor antigens were prepared using a modification of the methods of Bloome et al (1969) and Halliday (1972) We placed the freshly cleaned and minced tumor tissue in medium 199 (Institute of Immunology Zagreb) which contained 100 IJ of penicillin/ml and 60 μg of streptomycin/ml This was homogenized in a water bath in a Sorvall machine for 45 min at 1 000 revolutions/min The suspension was then transferred to a test tube and centrifuged at 1 000 g for 30 min The sediment obtained mainly contained membranes and larger subcellular particles The supernatant was then centrifuged at 100 000 g for 60 min and the clear acellular supernatant was stored in 1 ml ampules at −20°C until use

Out of the 16 examinees with laryngeal cancer tested with tumor antigens 7 responded positively and 9 negatively We considered a positive reaction to be cultures with more than 3% lymphocytes transformed by the antigen Out of the 7 with positive findings, 2 died shortly thereafter 4 are in good condition and 1 is in poor condition The difference between the findings of the PHA test and the lymphocyte number in the two groups of patients was insignificant Based on these results we cannot predict the outcome of the disease in laryngeal cancer by determining the in vitro reaction to tumor antigens More than likely it will be necessary to determine the specific antigen for each patient. This represents a problem however considering the testing procedures involved and the expense of preparing these antigens

HUMORAL AND CELLULAR IMMUNITY

It is considered that bone marrow and the fetal liver in man are the most likely sites for producing B lymphocytes The majority of B lym-

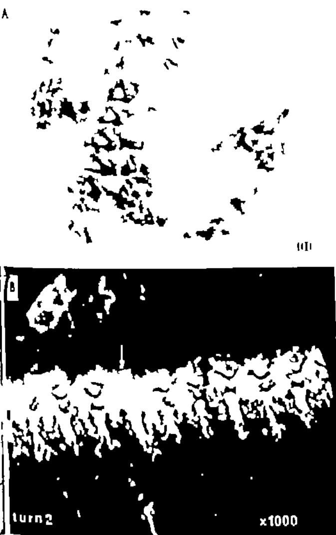


Fig. 5. (A) A surface preparation of guinea pig cochlea showing the normal regular pattern of the outer hair cell cilia (Phase contrast, 1000). (B) Cochlear irradiation with 100 milliwatts resulted in shorting and distortion of the cilia, and some hair cell losses in the inner most row of the outer hair cells (P.C. 1000). *ihc* inner hair cells, *ohc* outer hair cells; *p* pillar cells.

round window ultrasonic treatment of Meniere's disease was sufficient to initiate pathological change.

Following vestibular irradiation changes in cell structure were observed in a restricted region of the basilar membrane proximal to the round window. The resulting impairment of cochlear function would involve the response to high frequencies above the range of what is considered to be practical hearing (Von Bekesy 1960, Stevens et al. 1935). The same ultrasound irradiation regime has produced no deleterious change in the cochlear microphonic response to stimulus frequencies up to 8 kHz (Barnett 1979).

The results of cochlear irradiation demonstrated progressive cellular degeneration whereby the extent of immediate structural changes increased after a post irradiation period of latency during which cellular necrosis became histologically discernible. The immediate histological changes seem to be due to ultrasonically induced cellular agitation rather than a thermal mechanism as they have

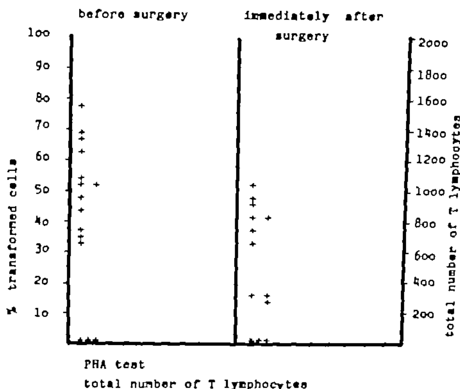


Fig 3 PHA test and total number of T-lymphocytes lowing surgery

histologically examined the tumor and its surrounding tissue to check local lymphocytic infiltration A positive correlation between the size of lymphocytic infiltrate and patient survival was found The 1–5 year survival rate in patients with modest or good infiltration of lymphocytes was up to 70% but only 40% in those with small infiltration The survival rate was down to 25% in those with T_2 tumo regardless of the type of treatment they ceived if lymphocyte infiltration was small patients with larger T_3 and T_4 tumors the s vival rates were over 50% in those with pc tive DNCB reactions and good infiltration a up to 100% in those with positive PHA fi ings and strong infiltration Sala & Fert

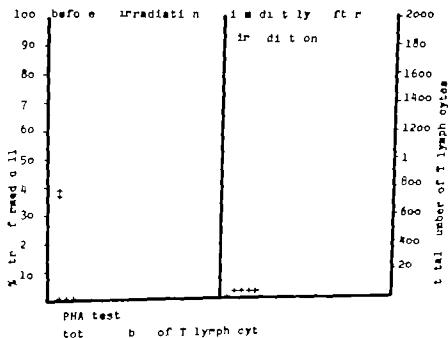


Fig 4 PHA test and total number of T-lymphocytes following irradiation

Acta Otolaryngol 89· 433–439 1980

FATIGABILITY OF THE STAPEDIUS REFLEX IN INDUSTRIAL NOISE

Roland Nilsson Erik Borg and Gunnar Lidén

From Health and Medical Care Department Gotaverkens Företagshälsovård AB Göteborg Department of Physiology II and Department of Audiology Karolinska Institutet Stockholm and Department of Audiology and Otolaryngology Sahlgren Hospital University of Göteborg Göteborg S eden

(Received April 19 1979)

bstract Normal hearing subjects were monaurally posed to 30 min of tape recorded shipyard noise (97 dB A) hich is characterized by a variable temporal structure. The stapedius muscle activity was continuously recorded in the opposite ear as change of the ear's acoustic impedance. The reflex function was, in addition, assessed stimulus-response curves obtained before and, at arious times after exposure. A slight reflex fatigue was bserved, together with a parallel shift of the stimulus-response curve (average 4 dB). The recovery was slow nd not complete even 10 min after the end of the exposure. The individual variability was large. For 5 of the subjects the exposure was repeated at later session and he individual degree of fatigue was found to be largely roducible. The present results suggest that the stapedius eflex might play role in the clinical picture of noise nduced hearing loss.

Much interest has been focused on the stability and decay of the stapedius reflex response o constant pure tone stimuli and noise signals Kato 1913 Werställ 1958 Dallos 1964 Djupesland et al 1966 Johansson et al 1967 Tietze 1968 Coles, 1969· Kaplan et al 1976 Lutman & Martin, 1978 Wilson et al. 1978) These studies all show very rapid adaptation of the response It is however also known hat a reflex response which has decayed will reactivate after even a very short pause (Metz, 1951 Wersall 1958 Borg & Ödman 1979) or after a change in intensity or frequency Gaevenes & Söhoel 1966) The sound usually resulting in damage to the inner ear—industrial noise—varies with respect to frequency and ntensity It is therefore difficult to predict from available data how the stapedius muscle acts during a workday in an industrial noise environment and thereby the possible role of the reflex in noise induced hearing loss The presently prevailing opinion is that the muscle rapidly adapts (e g. Tonndorf 1976) but there are also some early observations (Lüscher 1930· Kobrak et al. 1941) which indicate that this might not be the case Lüscher observed the stapedius tendon through a perforation in the ear drum. He described a rapid adaptation upon continuous steady sound stimulation but a remarkably great resistance to fatigue in a more varied sound environment.

The aim of the present work was to study the stapedius reflex characteristics in a realistic industrial noise environment. Normal hearing subjects have been unilaterally exposed under laboratory conditions to a 30 min sequence of noise recorded in a shipyard. The stapedius reflex was continuously recorded in the contralateral ear and stimulus-response curves at 0.5 and 2.0 kHz were obtained before and at various times after the exposure Of particular interest were the time course of reflex recovery after the exposure and individual variability with respect to fatigue A preliminary report has been published (Borg et al. 1979*a*)

METHODS

The experiments were performed on 18 subjects aged 18 to 47 years. Their hearing thresholds were within 20 dB of ISO 1964 be

The present study was supported by grants from the Swedish Work Environmental Fund (ASF 78/111).

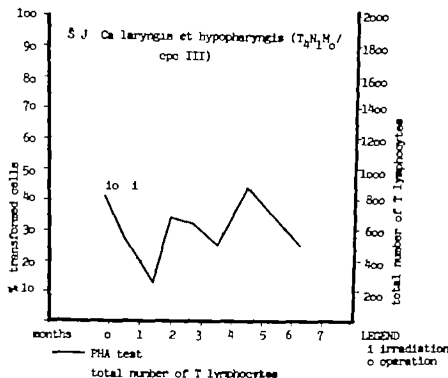


Fig 7 PHA test and total number of T lymphocytes in a patient following surgery and irradiation.

firm any difference (Fig 3) In another 18 patients we determined these values before and after treatment with irradiation and found that PHA percentages and the T lymphocyte number fell to almost half of their previous values (Fig 4) The difference was statistically significant ($P<0.01$) Fifteen patients (27 in total) received chemotherapy 14 after surgery and irradiation and 1 treated with chemotherapy only The percentages of the PHA test and the T-cell count were also compared before and after therapy and a statistically significant difference was found ($P<0.01$) (Fig 5) In two patients we followed the changes in the immunological status during combined therapy reduced the organism s immune reaction (Figs 6–7)

CONCLUSION

Although the immunological surveillance may be a factor in the development of a tumor we still have to consider it as just one among the variables interacting in the process of tumor destruction (Jurin) As clinicians we believe it absolutely necessary today to perform immune reaction tests in patients with laryngeal cancer If these tests are positive that is within the control range the prognosis is better This is particularly important in the local immune response to the tumor If the DNCB test proves to be negative this should not be considered a contraindication for surgery instead other immunological tests should be performed such as PPD PHA and the absolute number of T lymphocytes As the blood analysis is often necessary for following the course of the disease before and after therapy so is the control of the immunological status important for the evaluation of the organism s overall condition before or during the administration of various kinds of treatment such as irradiation and chemotherapy

RÉSUMÉ

On suppose que les tumeurs malignes apparaissent plus souvent chez les sujets dont l'immunité cellulaire est insuffisante L épreuve cutanée DNCB était positive 40% du nombre total du cancer laryngien au debut tandis que chez les sujets normaux la positivité de cette réaction cutanée est présente dans 85%–95% des cas Les lymphocytes T ainsi que leur produits – Lymphokines et les facteurs de transfert – jouent un rôle essentiel. Bien que les B – lymphocytes avec leurs anticorps humorant entraînent un développement tumoral plus rapide une interréaction entre les lymphocytes T et lymphocytes B a est

Procedures After otoscopic examination and hearing threshold determination, stimulus-response curves were determined for 0.5 kHz and 2.0 kHz pure tones During the 30 min noise exposure the contralateral response was continuously recorded. The balance of the impedance bridge was continuously controlled and the subject was instructed to swallow repeatedly After the end of the exposure the stimulus-response curves were again obtained according to the following schedule Immediately after the end of the noise 2.0 and 0 5 kHz; after 5 min. 2.0 kHz after 10 min. 2 0 and 0.5 kHz. Finally tympanometry and pure tone audiometry were repeated

RESULTS

The first and last minutes of the exposure noise were identical. This allowed the detection of even minor changes in reflex characteristics. Fig. 1 shows a typical example of such a sequence of recordings The upper graph shows a recording of the noise signal wherein the high intensity periods appear diffuse The middle graph shows the impedance change in the contralateral ear during the first minute and the lower graph shows the corresponding recording during the last minute after 30 min. It is seen that the impedance change well follows the variations in noise intensity It is especially notable that the impact noise elicits a series of responses which summate to give a background level of muscle activity with superimposed peaks The impedance change is altered to some extent after the 30 min exposure Only the lower level components are depressed, stronger sounds still give maximum responses

The stimulus-response curves obtained at various times post-exposure were compared with the corresponding curves obtained before exposure in order to quantify the alteration of the reflex and to establish the rate of recovery Fig. 2 shows typical stimulus-response curves before (continuous line) and less than one minute post-exposure (broken line) The stimulus-response curve after exposure is more or less parallel although shifted to the right and the maximum is unchanged A shift of this type is compatible with a selective suppression of the low level activity as shown in Fig. 1

In order to quantify the changes of the reflex the shift of the stimulus-response curve (B in Fig. 2) was measured and expressed as a function of sensation level (A=dB re reflex threshold Fig. 2) The suppression of the stapedius reflex after 30 min noise exposure is described in this way in Fig 3 Fig. 3 A shows shifts at 2.0 kHz less than one min post-exposure and Fig. 3 B shows shifts 0.5 kHz about 2 min after exposure Each subject is shown by a thin continuous line and the mean value ±s e is shown by the heavy line It is seen that the variability is appreciable Some subjects exhibit an improvement of response others suffer more or less pronounced fatigue The reflex depression was significant ($P<0.05$) only at 2 0 kHz (5 and 10 dB re reflex threshold) The average shift is largely independent of the sound level which is indicative of a parallel shift rather than a change of slope of the stimulus-response curve In conclusion there is a small but significant reflex fatigue following an exposure to the 30 min sequence of industrial noise

To investigate the remarkable spread of data seen in Fig. 3 five subjects were reexposed to the same noise from a few days to one year later and retested

Fig. 4 shows pairs of curves for each subject representing alterations of reflex properties based on 2.0 kHz pure tone stimulation less than one min post-exposure The individual mean values are indicated to the right in the graph. It is seen that one subject has consistently large shifts in one direction while another one consistently showed a potentiation of the reflex (negative values) The remaining subjects show intermediate reactions Although no quantitative estimation of reproducibility can be made the results presented

GENERAL DISCUSSION

T. Palva – Kastenbauer – Friedmann – Surján Jr. – Albegger – Krajina – Zechner – Jongkees – Arnold – Wersäll – Naumann

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Professor Gunnar Lidén, M.D.
Dept. of Audiology
Sahlgrens Hospital
S-415 45 Göteborg, Sweden

Acta Otolaryngol 89 204–213 1980

THE NORMAL HUMAN MAXILLARY SINUS MUCOSA

An Electron Microscopic Study

Hussein H Toppozada and Mamdouh A Talaat

From the E.N T Dept Faculty of Medicine Alexandria Egypt

Abstract The fine structure of the maxillary antral mucosa was studied in 5 cases The findings revealed the similarity of the nasal and paranasal sinus mucosae Certain differences were recorded denoting relative diminished activity of the seromucinous glands diminished exchange between tissue fluid and blood diminished number of cilia, delicacy and loosening of the epithelium Thus in inflammation there is lower resistance of the sinus mucosa to infection and easier penetration for microorganisms requiring early systemic administration of antibiotics with postural drainage and antral irrigation subsequently in treatment

The maxillary sinus originates as an outpouching of the nasal mucosa of the infundibulum in the embryo of about 32 mm CRL i e 9th week of intra-uterine life (Keith 1949) It expands progressively occupying more of the future maxillary body The lining epithelium is derived from the olfactory epithelium of the middle nasal meatus and therefore undergoes the same pattern of differentiation as does the respiratory segment of the nasal epithelium proper (Bhaskar 1976)

Light microscope studies have established the similarity of the nasal and paranasal sinus mucosae (Bradbury 1973 Leonhardt 1977) though the following differences have been reported the mucosa is thin and less specialized the epithelium is lower and contains fewer goblet cells a basement membrane is for the most part not visible the glands are fewer and smaller and the venous erectile plexus is absent (Arey 1971)

The ultrastructure of the normal human nasal mucosa has been described by various authors the epithelium by Tremble (1955 1962 1967) Rhodin (1957 1959 1963) Spoendlin (1959) Ricci & Gasparini (1960 1964) Burian & Stockinger (1963) the vascular bed by Caura & Hinderer (1969) Cauna (1970) the seromucinous glands by Toppozada & Gaafar (1973) Of the normal human maxillary sinus mucosa, only the ultrastructure of the secretory cells of the submucosal glands has been investigated (Vidić & Tandler 1976)

The aim of the present work was to complement the ultramicroscopic study of the human normal antral mucosa and to record any differences from that of the nasal mucosa.

MATERIAL AND METHOD

Five patients (3 males and 2 females) aged 20–35 years were chosen They were undergoing dacro-cystantrostomy surgery but had normal healthy maxillary sinuses. The samples were taken under local surface anaesthesia without adrenalization from the sinus mucosa of all walls The tissues were fixed in 4% glutaraldehyde solution followed by 1% osmium tetroxide solution buffered at pH 7.4 with phosphate buffer The tissue blocks were embedded in an Araldite mixture (Luft 1961), and cut with the automated ultramicrotome 111 type 8801 A (LKB) The sections were stained with 5% uranyl acetate in 1% acetic acid followed by 0 04% lead citrate Electron micrographs were prepared in a Siemens Elmiskop I

RESULTS

The ultrastructure of the maxillary sinus mucosa revealed a similarity of the fine details to

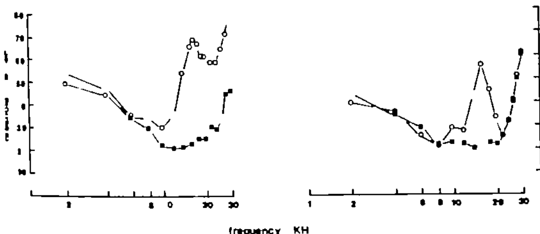


Fig 2 T types of hearing loss (■ pre-lesion, O post-lesion) induced when the organ of Corti is mechanically disrupted The type of loss shown by GP71 is termed notch, while GP65 shows in addition a loss in N sensitivity in the higher frequencies.

Puncturing of the basilar membrane and in some cases Reissner s membrane was carried out in 13 animals Twelve of these (one was not completely measured on this occasion GP79) showed an immediate loss of sensitivity in the form of a high frequency notch The maximal loss of the notch immediately post lesion (within 5 min) occurred at frequencies ranging from 13 to 20 kHz with a mean of 16.5 kHz (S D 2 0) The magnitude of the loss ranged from 9 to 56 dB with a mean of 28.5 dB (S D 15 6)

No significant changes from pre-lesion thresholds up to 8 kHz were evident in the post-lesion electrocochleogram for all animals In the eight ears where no loss occurred on opening the scala tympani the mean loss after puncturing was 1 6 dB (S D 13 1 range 9–56 dB) and occurred at 13–20 kHz (mean 16 6 kHz, S D 2 5)

In four ears showing a loss on opening scala tympani the additional effect of puncturing the basilar membrane was studied Mean loss before puncture was 35 0 dB (S D 10 4) at 16.3 kHz (S D 0 5) and after puncture 4_1 dB (S D 10 7) at the same frequencies Thus the additional effect of puncturing the basilar membrane on the hearing loss was 7 1 dB (S D 2.7)



Post recovery Electrocochleograms

Recovery period varied from 4 to 43 days. Post-recovery electrocochleograms in animals showing initial hearing loss showed improvement in post-lesion threshold in all but 2 of the animals Three of the animals had a recovery period of 4–8 days 8 had recovery periods of 17–21 days and 9 had recovery periods of 27–43 days No significant differences occurred in the amount of recovery between these three groups

The 4 animals which showed a loss on opening the scala tympani and in which the basilar membrane was not punctured showed a very small recovery (mean 2.5 dB S.D 5.3 dB) The eight ears which were punctured and did not have a loss on opening scala tympani showed slightly more pronounced recovery (mean 7 9 dB S D 12.0 dB) Three of the animals included in this group showed complete recovery (Fig. 4) The 4 animals which showed loss on opening scala tympani—and in addition the basilar membrane was punctured—

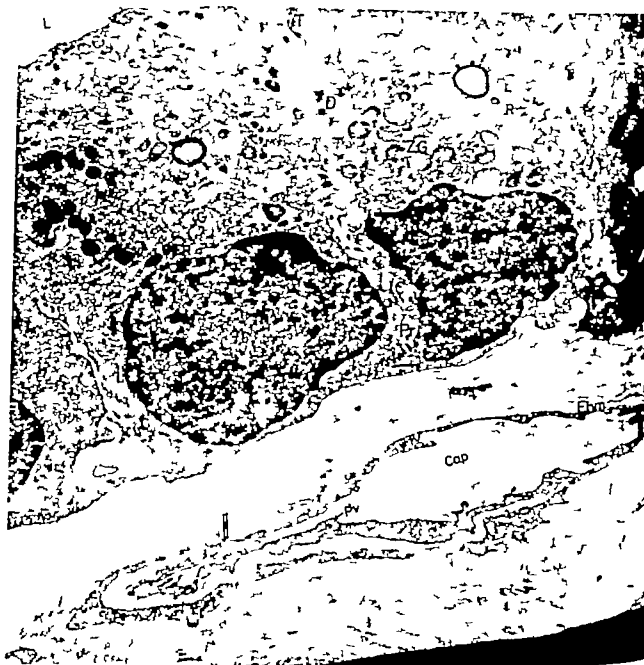


Fig 2 Electron micrograph of a serous gland showing part of the lumen (*L*) intercellular canaliculi (*IC*) dilated near the bases of the cells with finger-like cytoplasmic processes (*P*) many desmosomes (*D*) and terminal bars (*T*) The cells contain few zymoge granules (*ZG*) fewer free ribosomes (*R*) granular endoplasmic reticulum (*GER*) small mitochondria (*M*) Golgi apparatus (*G*) and nuclei (*N*) A periglandular capillary (*Cap*) is seen with few micropinocytotic vesicles (*P*) endothelial basement membrane (*Emb*) with pores (↖↖) and pericyte (*Pi*) ×96.5

mucous secretory cells had fewer microvilli on their luminal surfaces The intercellular canaliculi were dilated near the bases of the cells with finger like cytoplasmic processes extending in between them The canaliculi tapered off and became narrow as they approached the lumen showing more junctional complexes in the form of desmosomes and terminal bars

In serous cells (Fig 2) the zymogen granules were fewer in number and paler in density though variations in their densities and sizes existed A few rounded or oval empty spaces having a definite limiting membrane were seen

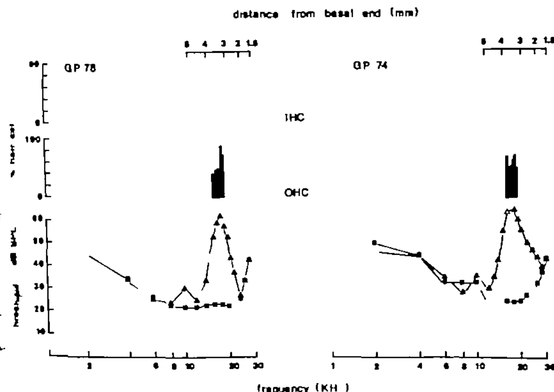


Fig 4. Upper portion of figure shows hair cell loss (both OHC and IHC) plotted as function of distance from basal end of basilar membrane. Hair cell counts are expressed as percentage loss per 100 µm (or groups of 10 inner hair cells) Lower portion of figure shows the N lectro-cochleogram (Δ, post-recovery N thresholds). The relationship between the N thresholds and spatial location of the lesion is explained in the text GP 74 and 78 both lost only outer hair cells and had bearing loss on opening the scala tympani the organ of Corti was not pierced

venience in estimating the size of the lesion abnormal hair cells were scored as absent. Neither of the 2 sham-operated guinea pigs showed hair cell losses or structural abnormalities in the region of the cochlea in question

The width of these lesions varied from 70 µm to 1 1 mm with an average width of 486 µm. In all but 4 animals both inner and outer hair cells were lost or showed severe abnormalities, but in every case more outer hair cells (OHC) were lost than inner hair cells (IHC) OHC loss generally extended both basally and apically beyond the region of IHC loss.

In the 4 animals in which a loss of N sensitivity occurred on opening the scala tympani, hair cell damage was restricted to the outer hair cells. The loss of OHCs was greatest in the third row and decreased dramatically towards the first (innermost) row All inner hair cells were present and appeared structurally normal in these animals. An example of SEM results from a lesioned cochlea is shown in Fig 5 A complete description of the morphology of these lesions is included in a paper in preparation

Relation between Post recovery Electrocochleograms and Cochlear Morphology

Examples of the type of correlation found between post-recovery electrocochleograms and hair cell loss are shown in Figs 6 and 7 In these figures the anatomical lesion has been plotted as a function of distance from the basal



Fig 4 Electron micrograph of a muccous gland showing lumen (*L*) lined with few microvilli (*Mv*) intercellular canaliculi (*IC*) finger-like cytoplasmic processes (*P*), many desmosomes (*D*) and terminal bars (*T*) The cells contain large pale mucigen granules (*MG*), fewer free ribosomes (*R*) granular endoplasmic reticulum (*GER*) small mitochondria (*M*) Golgi apparatus (*G*) and nuclei (*N*) ×5775

packed droplets showing diminished activity with fewer free ribosomes and smaller mitochondria and Golgi apparatus

III *The blood vessels*

A *The capillaries* (Fig 5) There were fewer endothelial cytoplasmic microvilli extending into the lumen The endothelium contained fewer and smaller micropinocytotic vesicles The majority were non-fenestrated except for the periglandular capillaries (Fig 7) The endothelial basement membrane was frequently porous and in some areas consisted of several incomplete layers This was more evident in the periglandular capillaries There were fewer collagenic fibres surrounding the basement membrane

B *The venules* (Fig. 6) The endothelium

mission electron microscopy (TEM) may reveal ultrastructural abnormalities within the actual cell body. No studies of this nature were carried out in this experiment.

DISCUSSION

The sequence of events following mechanical disruption of the organ of Corti and subsequent degeneration of the receptor cells is as yet obscure. Introduction of a 20 μm glass probe through the cochlea partition in this study produced loss of hair cells for distances of up to 1.1 mm. One possible mechanism which may result in hair cell degeneration is a mixing of the cochlear fluids perilymph and endolymph. Disruption of the integrity of both Reissner's membrane and the cochlea partition in other studies has produced loss of receptor cells similar to that found in this series of animals (Lawrence 1966, Duvall & Rhodes 1967, Duvall et al. 1969). In our experiments N_1 thresholds were not followed for more than 30 min after piercing the organ of Corti and consequently it was not possible to assess the final extent of the hearing loss until post recovery electrocochleograms were measured. Because the final lesion was in all cases much more extensive than the size of the probe, pure mechanical disruption of the organ of Corti seems a remote cause of the final loss of the receptor cells. In any case this method has enabled spatially restricted, reproducible loss of hair cells to be correlated with the resultant deficiencies in the N_1 electrocochleogram.

The use in this study of SEM rather than serial sectioning and surface preparation (Engström et al. 1966) allows for a greater degree of accuracy in assessing the morphological condition of the organ of Corti. In serial sectioning, as Bredberg & Hunter Duvar (1975) have pointed out, every 10th section is generally stained and inspected which means that small lesions may possibly be missed. No such inaccuracy exists in our data. Lesions as small as 32 μm (Fig. 5*a*) incorporating only 8 OHCs in area are clearly visible and the absence of only one hair cell is easily detectable. In addition the high resolution of SEM will reveal surface structural abnormalities which may go undetected with light microscopy. We have assumed that obvious abnormalities in cuticular plates and stereocilia imply abnormal functioning of the hair cell.

Several important points emerge from our results. Firstly, the excellent correlation between location of maximum hair cell loss and frequency of maximum loss of N_1 threshold sensitivity provides an additional validation of the place-frequency map of the guinea pig cochlea, even though our lesions were confined by necessity to a fairly restricted region of the basal cochlear spiral. The most complete place frequency maps have hitherto been derived from basilar membrane mechanical measurements (Wilson & Johnstone 1975) and from spiral ganglion recordings (Robertson & Manley 1974, Johnstone 1977), both methods showing substantial agreement. From data provided by Wilson and Johnstone (1975) the equation

$$f = 45 e^{-0.33x} \qquad (1)$$

can be derived, where f is the cut-off frequency for the basilar membrane tuning curve and x is distance from the basal end of the basilar membrane. Excellent agreement was found in our present data between the frequency of maximum loss in the N_1 electrocochleogram and the location of hair cell loss when these were aligned according to the above equation. Thus eq. (1) appears to reflect accurately the spatial representation of frequency in auditory nerve fibres. The present N_1 data, together with recent spiral ganglion cell recordings (Robertson et al., in preparation) from our laboratory suggest that the primary nerve fibres at a given location respond best to the frequency corresponding to the high frequency cut-off rather than the peak frequency of the basilar membrane tuning curve at that location (Wilson & Johnstone 1975). This may have im-



Fig 6 Electron micrograph of a venule showing lumen (*L*) with red blood corpuscles (*RBC*), thin endothelial cell (*E*) containing few pinocytotic vesicles (*Pv*) single continuous endothelial basement membrane (*Ebm*) and muscle cells (*MC*) in the tunica media. ×5775

DISCUSSION AND CONCLUSION

Although the electron microscope revealed the great similarity of the ultrastructure of the maxillary sinus and nasal mucosae many differences could be identified which help increase our knowledge about this pneumatic cavity

There was diminished activity of the seromucinous glands as evidenced by

(*a*) Their scarcity

(*b*) The diminished number of secretory granules which appeared pale and absent from the lumen and the diminished activity of the cell organelles

(*c*) As the uptake of serum constituents through the basal lamina into the glands decreased the intercellular canaliculi were dilated near the bases of the cells towards the

ous authors (Kiang et al 1976 Dallos & Harris, 1978 Robertson & Johnstone 1978) that the outer hair cells may in some as yet obscure way be necessary for the normal functioning of the afferent nerve fibres which innervate the inner hair cells.

ACKNOWLEDGEMENTS

This work was supported by grants from the Australian Research Grants Council and by the Swedish Medical Research Council, project no 3542. G. Bredberg was supported by a Rank Foundation Visiting Professorship. D. Robertson is Queen Elizabeth II Fellow A Cody is a Commonwealth Postgraduate Student.

ZUSAMMENFASSUNG

Kleine getrennte Verletzungen wurden im Cortischen Organ der Meerschweinchencochlea erzeugt, indem feine Sonden gebraucht wurden, um eine direkte mechanische Verwundung hervorzubringen. Der elektrophysiologische Zustand der Cochlea wurde durch N Elektrocochleographie eingeschätzt und der Verlust an Receptorzellen durch Abtastung mit Elektronmikroskop bestimmt.

Die Hauptbefunde waren 1) Vortreffliche Übereinstimmung zwischen dem Fokus von Haarzellenverlusten und der Frequenz von Hochstempfindlichkeitswechsel im N Audiogramm. 2) Die räumliche Ausdehnung der mechanisch bewirkten Verletzung scheint wichtiger zu sein als die Gesamtanzahl der verlorenen Haarzellen für die Bestimmung der Höhe des Verlustes an N_1-Empfindlichkeit. 3) Haarzellenverluste, die sich über nur 77 µm ausdehnten, konnten als bedeutsame Veränderungen der N Empfindlichkeit festgestellt werden. Diese Resultat betonen weiterhin die Genauigkeit und Brauchbarkeit des N_1-Elektrocochleogramms für die Einschätzung der Leistungsfähigkeit der Cochlea. 4) Verletzungen, die sich nur auf Verluste der äußeren Haarzellen beliefen, bewirkten auch markierte Erhöhungen der N Schwelle

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DISCUSSION AND CONCLUSION

Although the electron microscope revealed the great similarity of the ultrastructure of the maxillary sinus and nasal mucosae many differences could be identified which help increase our knowledge about this pneumatic cavity

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(*a*) Their scarcity

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Fig 4 Wedge-shaped replacement of the periost by sclerotic portion advancing by fingers. ×82.

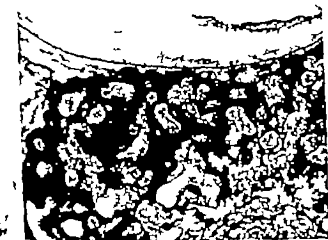


Fig 5 Replacement of endost, in rosary formation, by spongiotic focus. 82.



Fig 6. Replacement of endost by focus rich in fibrous component. 52.

sue fluid and the blood as *evidenced by the* reduced number and size of the endothelial cytoplasmic microvilli and micropinocytotic vesicles the absence of gapping of the interendothelial cells and preponderance of the non fenestrated capillaries *Fenestrae occur where there must be a* particularly active and unimpeded exchange between tissue fluid and the blood Hence in maxillary sinus inflammation early systemic administration of antibiotics is advisable as its diffusion towards the epithelium will help resolution *Antibiotic concentration in the tunica will be less* effective should the micro-organism invade deeper layers and may necessitate higher doses

By way of ciliary beating the mucous blanket lining the epithelial surface moves generally from the sinus interior in the direction of the ostium towards the nasal cavity In sinusitis clumps of exudate are occasionally too thick and heavy to be readily compressed through the ostium and obstruct the opening or drop back down to the floor (Van Alyea 1970) The diminished number of cilia in the self-cleansing mechanism helps this cycle The shape of the maxillary sinus as a four sided pyramid with a quadrilateral base which *is facing medially toward the nasal* cavity with an ostium at the level of the middle nasal meatus and an apex pointing laterally toward the body of the zygomatic bone adds to the difficulty of the self-cleansing process Hence the importance of a postural drainage such as the Proetz position and the side position of Parkinson early in the treatment and antrum irrigation later on to remove a large number of micro-organisms from contact with the epithelium having impaired resistance

The absence of cavernous venous tissue excludes the maxillary sinus as playing any important part in warming of the inhaled air but the mucous blanket passing to the nose helps in the process of humidification

RÉSUMÉ

L ultrastructure de la muqueuse normale d sinus maxillaire fut étudiee dans cinq cas. Les données montraient *la similarité des muqueuses nasalé et celles du sinus pa*-nasal Certaines différences furent notées [illegible] diminution relative de l'acti ité des glandes séromuqueuses une diminution du nombre des cils, une [illegible] des échanges entre le fluide ambiant et le sang [illegible] que l'aspect frêle de l'épithélium et son relâchement En cas d'inflammation la diminution de la résistance de muqueuse du sinus contre l'infection alliée à une pénétration facile des microbes nécessite l'administration précoce d'antibiotiques avec un drainage postural et plus tard au cours du traitement l'irrigation de l'antre maxillaire

ZUSAMMENFASSUNG

Die feine Struktur der normalen Kieferhöhlenmucosa wurde an funf Patienten untersucht Die Ergebnisse zeigten große Ahnlichkeit zwischen nasalen und paranasalen Mucosae Bestimmte Unterschiede zeigen auf relative abnehmende Aktivität der seromukösen Drüsen, abnehmenden Austausch zwischen Gewebeflüssigkeit und Blut, abnehmende Zahl der Cilia, Verfeinerung und Erlockerung des Epithelums Somit geschieht bei Inflammation geringere Resistenz des Sinus mucosa gegenuber Infektion und leichtere Penetration für Mikroorganismen, das heißt es wird eine zeitige systemische Verabreichung von Antibiotika mit posturaler Dränage und antraler Irrigation nach der Behandlung erforderlich.

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Acta Otolaryngol 89: 459–464 1980

THE DIAGNOSIS OF NEGATIVE MIDDLE EAR PRESSURE IN CHILDREN

The Accuracy of Symptoms and Signs Assessed by Tympanometry

T Lildholdt, J Courtois B Kortholm J W Schou and H Warrer

From the Department of Otolaryngology Vejle Hospital Vejle Denmark

(Received April 19 1979)

Abstract Over period of one year we studied 357 children at random in order to determine whether or not diagnosis of negative middle ear pressure can be made reliably without using tympanometry Each child was tested repeatedly by screening audiometry at 20 dB and by tympanometry and was examined once by otoscopy Information about upper respiratory tract infections, feelings of oppression in the ears, and the parents opinion of the child' hearing ability were obtained from questionnaires Otoscopy correlated poorly with the tympanometry while screening audiometry and the parents opinion of the child' hearing ability were more reliable measures It is concluded that tympanometry is an absolutely necessary tool in the clinical diagnosis of negative middle ear pressure

Negative middle ear pressure which is the result of an impaired function of the Eustachian tube occurs very frequently in children (Renvall et al 1978 Fiellau-Nikolajsen et al 1977). If it is persistent secretory otitis media (SOM) may develop a condition which is characterized by a nonpurulent middle ear effusion Because the results of today s treatment of SOM are not satisfactory as stated by Tos & Poulsen (1976) early diagnosis and treatment of this problem if possible before the effusion has developed would be desirable

Impedance audiometry or tympanometry has gained widespread use in otology as a mean of documenting the middle ear pressure objectively The frequency with which this technique is used seems to vary considerably among otologists, and its use is very limited among colleagues in other specialties such as pediatricians and family doctors. Thus some doctors may claim that the clinical diagnosis of middle ear pathology (i e of negative pressure) can be made with sufficient reliability without tympanometry

In order to evaluate the truth of this assumption it is necessary to examine what is meant by a diagnosis For this purpose the elements of the clinical diagnosis may be defined as symptoms and signs Symptoms are those concomitants of the disease or clues to the disease about which the patient can give positive statements while signs may be observed or measured by the physician Thus the clinical diagnosis may be analysed by evaluating each symptom and sign separately but without analysing the complex interactions of all elements together

This principle was applied to an epidemiological study conducted during 1977 of negative middle ear pressure in a randomly selected group of children The signs of middle ear disease were evaluated from tympanometric audiometric and otoscopic observations and some corresponding symptoms of middle ear pathology were obtained from questionnaires answered by the parents

METHODS

The study involved children from six schools in Vejle which is an industrial town of about 50 000 inhabitants From 16 classes a total of 357 children were selected all of whom were

Acta Otolaryngol 89 214–221 1980

IgA TRANSPORT MECHANISM THROUGH THE HUMAN NASAL MUCOSA AN IMMUNOENZYMATIC ULTRASTRUCTURAL STUDY

S Crifò and M Russo

From the Department of Otorhinolaryngology and Audiology and Department of Pathology University of Rome Italy

Abstract The role of secretory IgA antibodies in the antiviral and antibacterial defense of the higher respiratory tract has been widely demonstrated and the IgA determination in the nasal secretion has become of the highest value in the clinical study The mechanism by which IgA reaches nasal secretion through the mucosa is still a matter of discussion and neither hypothesis put forward by Tomasi (i e free filtration through the epithelic-connectival interstitium or transcellular transport *plus* active secretion) has so far been fully demonstrated. By using a horseradish peroxidase labeled IgG directed against human IgA we have performed with suitable controls a semi-quantitative study on semi-thin and thin section of the IgA behaviour at the level of human nasal mucosa We have shown that IgA made up and secreted in the connectival interstitium by specific plasma cells, would be taken up by endocytosis by epithelial cells where it can be seen especially in the Golgi apparatus and in the large secretion granules. Since in the intracellular transport of pinocytic vesicles actin microfilaments and microtubules undoubtedly play a major role with ATP utilisation further studies using either physical factors (e g exposure to cold) effecting ATP utilisation or chemical ones (e.g. cytochalasin B and colchicine) depolymering respectively microfilaments and microtubules should be performed in order to bring further proofs in favor of the active transport IgA mechanism suggested by our present investigations

Immunoglobulins A constitute the *major share* (80%) of immunoglobulins present in the secretions on the lining epithelium of the mucous membranes and therefore they are regarded as the first immunological barrier specific against viruses bacteria and other antigens (Tomasi et al 1972)

Their total or partial deficiency is associated with various chronic (or acute recurrent) infectious diseases (South et al 1965) It is long since known that IgA are synthetized in monomeric form (7S) by plasma cells in the lymphatic tissues and above all by those in the subepithelial connective tissue of mucous membranes The monomeric form (7S) represents 85–95% of serum IgA These have a tendency to polymerize as dimers (10S) trimers (13S) tetramers (15S) and pentamers (17–18S) Plasma cells also produce the *J component* a protein of 23000 MW present in polymers polymers represent only a minor share of serum IgA whereas they are almost all secretory IgA In detail 80–90% are dimers 15–10% are polymers (Tomasi et al 1972)

It has also been shown that secretory IgA apart from the J component have another specific protein component 55000–70000 MW the *secretory component* that they receive when passing into secretions fluid (Heremans 1974 Tomasi et al 1977)

This different structure of serum IgA as compared to secretory IgA and the preferential concentration of polymers into secretions has made one think about a specific transport mechanism from the blood or from the interstitial space of subepithelial connective tissue into the various secretions through the epithelium

Tomasi et al (1968) have proposed two mechanisms from the very beginning the first one includes the passage of IgA through cellular junctions as far as the surface of the epithelium where they are found in the secretions This mechanism however would not explain the difference between the dimeric form in the serum and that in the secretions and therefore the presence of the secretory component Furthermore it was later (Pap-

have analysed the prognostic abilities of more specialized methods of otoscopy such as the use of the operating microscope (Axelsson & Lewis 1976) which showed a very high accuracy rate. Roeser et al (1977) compared the assessments of an experienced otologist and a pediatrician each of whom examined the children with a pneumatic otoscope and impedance measurements. They found poor agreement between the results of otoscopy and impedance measures as well as poor inter-examiner agreement.

The best relative liability was found concerning the parent's assessment of the child's hearing ability and concerning the results of screening audiometry, however the sensitivity of these assessments was only about 25%. The reports in the literature on screening audiometry are difficult to compare as the criteria for abnormality vary. In general the results from our study seem to be in agreement with those of most other investigations (Berry et al. 1975, Brooks, 1973).

Only about one-fourth of the measured pathological middle ear pressures were accompanied by positive symptoms and signs. This appears from Fig. 1 as the 3.7% of the measurements with *both features* vis-à-vis the total of 15.4% of the measurements that showed pathological middle ear pressures. So this simplified analysis shows that the symptoms and signs which we evaluated are not good prognosticators of negative middle ear pressure below −160 mmH$_2$O, besides being a possible precursor of SOM, a pressure in that range should be considered a possible hearing handicap (Lildholdt et al. 1979). Accordingly the recognition of a negative middle ear pressure is important. This study shows the limited value of simple otoscopy in this diagnosis. This is especially significant because of the importance attributed to this procedure by non-otologists. In fact, more reliable information may be obtained by asking parents about their child's hearing ability or by recording a screening audiogram than may be obtained by otoscopy.

This study confirms the conclusion of many other investigators that impedance audiometry is an absolutely necessary tool in the clinical diagnosis of negative middle ear pressure.

ACKNOWLEDGEMENTS

The authors are greatly indebted to the staff of the Institute of Social Medicine, University of Århus, which placed their computer facilities at our disposal for the statistical analyses.

Erdem I. Cantekin, Ph.D., Director of Research, Department of Otolaryngology, Children's Hospital of Pittsburgh, Pittsburgh, Pa., USA provided great help in preparing the manuscript.

This work was supported by grants from "Den Lægevidenskabelige Forskningsfond for Vejle Amt" and "Åge Holm og Hustrus Mindefond" ved Vejle Sygehus. Statistical consultation was provided through the Danish Medical Research Council.

ZUSAMMENFASSUNG

In einer Periode von einem Jahr haben wir 357 zufällig ausgewählte Kinder studiert, um herauszufinden, ob es möglich ist, eine zuverlässige Diagnose eines negativen Mittelohrdrucks zu stellen, ohne Tympanometrie zu verwenden. Jedes Kind wurde wiederholt durch Screening Audiometrie bei 20 dB sowie bei Tympanometrie untersucht und wurde einmal durch Otoskopie untersucht. Auskünfte über Infektionen in dem oberen Respirationsraum, das Gefühl eines Drucks in den Ohren und die Beurteilung der Eltern über das Gehörvermögen des Kindes wurden durch Fragebogen erlangt. Otoskopie bildete eine schlechte Korrelation mit Tympanometrie, während Screening Audiometrie und die Beurteilung der Eltern über das Gehörvermögen des Kindes ein zuverlässiges Maß ausmachte. Daraus folgert, daß Tympanometrie ein absolut notwendiges Gerät in der klinischen Diagnose eines negativen Mittelohrdrucks ist.

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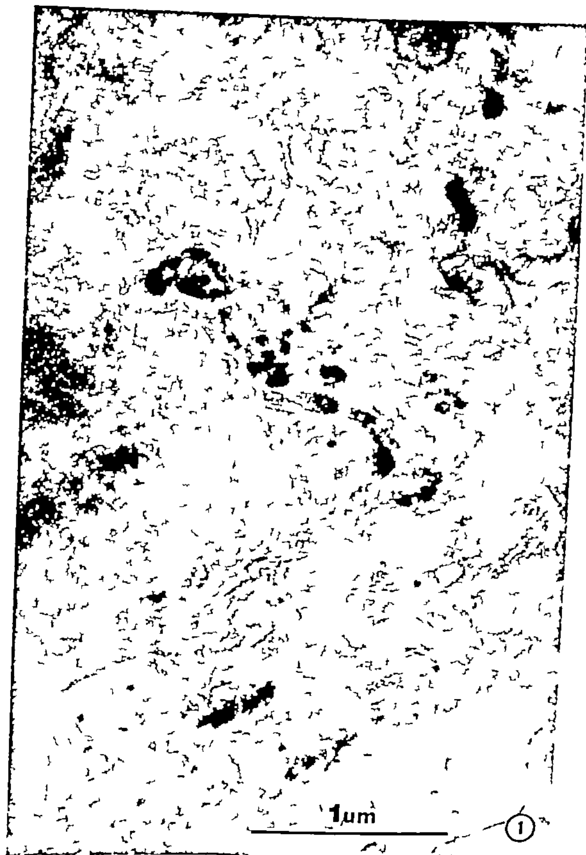


Fig 1 Human nasal mucosa treated with IgG anti-human IgA+HRP complex. The reaction product (DAB) can be seen in great quantity throughout the Golgi apparatus, both on the cisternae of dictosomes, and in the vesicles Unstained section ×45000



Fig 3 Scintigram of patient II presented according to Fig 1. Increased accumulation in the left mastoid region.

strated an abnormally high accumulation of the isotope corresponding to the right mastoid region and upper lateral part of the right external meatus which areas were left intact at earlier surgery (Fig 2) The patient was then irradiated with 60 Gy in fractionated doses. A second scintigraphy 2 weeks after the last irradiation dose showed an increase of the pathologic isotope accumulation The patient died from recurrent carcinoma of the ear and bronchopneumonia in March 1977

Patient II

A man aged 75 without history symptoms or signs of chronic otitis In 1961 a basal cell carcinoma of the skin developed behind the left auricle The tumour was excised, but from 1966 to 1976 local recurrences appeared and were excised five times In 1972 the periost of the mastoid process was excised in connection with the skin excision but the bone was left intact In July 1976 a 4×4 cm tumour was palpated behind the ear under the skin and against the bone Histology demonstrated a recurrence and the histopathological diagnosis was changed to squamous cell carcinoma. Radiography did not demonstrate any destruction but tomography demonstrated a destruction of bone under the tumour probably not explained by the excision of periost in 1972.

Scintigraphy was performed on the same day as the tomography and revealed an abnormally high radionuclide accumulation in the left mastoid region corresponding to the tumour recurrence and the destruction demonstrated by tomography (Fig. 3) The patient was irradiated with 40 Gy in fractionated doses. A second scintigraphy 2 weeks after the last irradiation dose demonstrated an increase in the pathologic isotope accumulation The patient was operated by radical mastoidectomy but in February 1979 again a recurrence was verified by histology

Patient III

A woman aged 72 with symptoms of chronic inflammation of the left middle ear since childhood with periods of excretion from the ear A chronic central perforation had been present at least for the last 5 years, but the ear had been dry during this period Since December 1975 excretion and slight pain had been present in the ear At examination the internal parts of the external meatus were filled with abnormal soft tissues possibly engaging the middle ear No symptoms of cochlear or vestibular engagement were present. Histologic examination of small biopsies revealed a highly differentiated squamous cell carcinoma. Ordinary radiography demonstrated a dense cell system and absence of bone details in the floor of the external meatus

Tomography of the ear also demonstrated the bone destruction of the floor of the external meatus The hearing bones could not be identified and the air space of the middle ear

Table I *Semiquantitative evaluation of osmium black reaction product in human nasal m*

	Anti-IgA antibody +HRP (%)	Anti-IgG antibody +HRP (%)	DAB only (endogenous peroxidase) (%)
% of cells with reaction product	100	30	28
Golgi apparatus, w/t reaction product	100	30	28
Secretory large granules w/t reaction product	80	3	3

somes were completely free of electron-dense osmium black products The same reaction product was neither visible in the smooth endoplasmic reticulum nor in the rough endoplasmic reticulum mithocondria or in the nucleus This could account for a scarce role of this organelle in direct transport mechanism of the secretory IgA

Both control specimens presented a few cells with reaction product (Table I) but it was localized in the Golgi apparatus or in its small vesicles in a very low quantity in comparison with the specimen treated with the complex containing the anti IgA antibody

The results we have attained show that IgA are preferentially located in quantities that can be evidenced with immunoenzymatic techniques in the Golgi apparatus and in the big secretory granules localized in the apical zone of epithelial cells of the nasal mucosa

Therefore it is likely that these two organelles are involved in the transport mechanism of IgA However it is not possible to exclude that even other organelles directly or indirectly intervene in this mechanism for the fact only that they do not have the electron-dense products of the peroxidasic reaction this could in fact be explained by the fact that IgA are scarcely concentrated here or that their turn-over (passage) is very rapid

On the other hand it has already been shown that RER or polysomes are responsible for the synthesis of the secretory component and it is also known that proteins synthesized by RER are started off to secretion through the Golgi apparatus (Russo 1977)

Therefore the diagram shown in Fig resents a possible sequence of events transport mechanism through the ep cells of the nasal mucosa, based on the attained by one of us and other auth analogous mechanism of polarized se and active transport (Russo 1977 Va sum & Russo 1979)

IgA molecules would be engulfed b docytosis from the interstitium at the pole of the epithelial cell in the dimenc the pinocytic vesicles migrate towar Golgi apparatus the vector of their r which indicates the cell polarity is deter by the architecture of membrane asso microfilaments (Clarke & Spudich 19 the Golgi region the pinocytic vesicles s meet the secretory component coming RER The functional tendency of Gol paratus to concentrate molecules *to b crete* made the IgA here immunological monstrable Indeed at Golgi apparatus IgA join to other secretory products formation of the large secretory granules

As an obscure point remains the reason IgA can be shown in the large granules with a medium-density homogeneous co whereas they have not been seen in the cous granules having double density (Fig is probable that the latter have not the a to fuse their membranes with the vesicles ing from pinocytosis and do not come in t with the secretory component

The mechanism we have propose analogous to that shown for other epith cells and in particular in the hepatic cell

tient VI

woman aged 40 suffering from external oti- of the left ear since February 1979 In July biopsy was taken demonstrating a medium-ly differentiated cancer of the ear and the tient was referred for surgery Radiography August 1979 demonstrated a slightly in-creased attenuation in the lateral part of the mastoid cell system of the affected ear Poly tomography revealed a destruction of the lower part of the external meatus as well as of the temporomandibular joint fossa and the an-terior part of the mastoid process

Scintigraphy demonstrated a definitely in-creased accumulation of ^{99}Tc^{m}DP in corre sponding regions but the area affected accord ing to the scintigraphic results was larger than according to the radiographic results (Fig 8)

The patient was operated one week later and the tumour was found to be growing ex tensively in the temporal bone and into the parotid region.

DISCUSSION

Five patients (I II III V and VI) with veri-fied squamous cell carcinoma of the ear and bone destruction demonstrated by tomo-graphy had abnormally high accumulation of ^{99}Tc^{m}DP in the temporal area. One patient with a basal cell carcinoma (IV) had no de monstrable bone destruction and a normal bone scintigram The abnormalities were dif ferently shaped in the gamma camera images which indicates a possibility of differentiating between different extensions of neoplastic growth in the temporal bone However in-flammatory as well as neoplastic processes in-fluence on bone accumulation of ^{99}Tc^{m}DP and abnormal accumulation patterns could have several explanations for instance neoplasia, inflammation or both (Greenberg et al., 1968 Galasko & Doyle 1972 Tofe et al 1975 Treves et al 1976 Bergstedt & Haverling, 1978 Bergstedt & Lind, 1978 Bergstedt &

The abnormal accumulation in the mastoid region of patient I (Fig. 2) might depend on inflammatory reactions provoked by earlier surgical excision of the auricula and the car tilaginous part of the external meatus or by the small biopsies taken later from this area. However biopsy demonstrated a recurrence of the tumour growth and the mastoid region and the bony part of the external meatus had been left intact by the surgeon It is therefore more likely that the abnormal accumulation was caused by a spread of malignant growth into the cortex of the mastoid process which also explained the destruction demonstrated by tomography

The abnormal accumulation in the mastoid region of patient II was most probably ex-plained by the tumour recurrence verified by histopathological examination which had changed the original diagnosis from basal cell to squamous cell carcinoma. However it cannot be excluded even if not probable that the pathologic uptake of isotope could be ex plained by the excision of periost of the region in 1977 and therefore this case also illustrates the necessity of careful analysis of scintigraph-ic findings in correlation to patient history in order to lessen the risk of false-positive diagnosis of neoplastic engagement of bone tissue

The engagement of the whole left pyramid of patient III indicates an influence on bone far beyond the radiologically demonstrable bone destruction of the tumour This patient had a chronic otitis of the left ear and the abnormal accumulation of the isotope might in part be explained by the chronic inflammatory reactions However no acute exacerbations had occurred during the last few years and so the abnormal accumulation was presumably explained by neoplastic influence on the bone Despite the macroscopically radical excision of the tumour area the patient eventually died from local recurrence The scintigraphy find-ings probably correlated better to tumour ex tension than did the tomography findings

The basal cell cancer tissue of patient IV

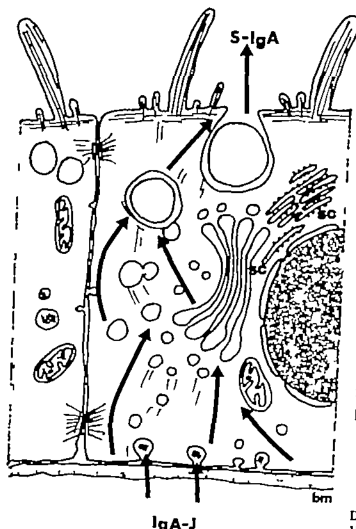


Fig 4 Diagram showing our hypothesis on IgA transport mechanism through the mucosal epithelial cell IgA–J IgA and J component arrive to the Golgi zone via pinocytosis S C secretory component is synthesised by endoplasmic reticulum IgA J-SC dimer-complex are formed at Golgi level in the large granules large granules are released at the cell surface (secretory pole) BM basal membrane

other polarized cells where an active and continuous flow has been shown from the interstitial space to the biliar duct (external space) via Golgi apparatus (Russo et al 1977)

Analogously this mechanism should be energy-dependent for IgA as well and strictly related to the integrity of the contractile internal system of the microfilaments and microtubules (Russo et al 1977)

Therefore it should be inhibited by all those physical or chemical stimuli which inhibit the utilisation of ATP or interfere with the function of microfilaments and microtubules de-polymerizing them (Russo et al 1976, Van Rossum & Russo 1979) Further studies are in progress to clarify the latter and other aspects In particular we are studying the effects of low temperature on IgA level in the secretions (it is in fact well known that respiratory infections have a higher rate during winter probably as a result of the weakening of the immunological barrier represented by secretory IgA as their mechanism of transport is certainly slowed down or inhibited by cold through a reduction of the utilisation of ATP and of local administration of cytochalasin B and colchicine (which depolymerize actin-microfilaments and microtubules respectively) (Van Rossum & Russo 1979) The IgA decrease in the secretions under such conditions would be a sufficiently strong argument itself in favour of the mechanism we have proposed

ZUSAMMENFASSUNG

Die Rolle der ausgeschiedene IgA Antikörper in der er
Verteidigung gegen Viren und Bakterien in den höhe
Atmungswegen ist schon allgemein demonstriert or
Wir halten die Dosierung in der Nasensekretion als
wichtige Probe für klinische rhinologische Unt
suchungen. Der Mechanismus, durch den IgA durch
Mukose in die Nasensekretion gelangt ist weiters un
kannt und beide von Tomasi aufgestellten Hypothe
(freie interstitiale Filtrierung oder transzellularer Tra
port und aktive Sekretion) sind bis heute nicht durch e
perimentelle Versuche unterstützt Mittels mit horse
dish-Peroxidase markierten IgG gerichtet gegen mensc
liche IgA und den geeigneten Kontrollen, haben w
semiquantitative Studien über das Verhalten der IgA
halbdünne und dünne menschliche Nasenmukose-Sch
ben ausgeführt Dadurch wurde bewiesen daß IgA, sy
thetisiert und in dem konnektivalen Zwischenraum vo
den spezifischen Plasmazellen ausgeschieden durch En
docytose von den epithelialen Zellen wo sie besonders in
Golgi und in den großen Sekretionsgranulen erscheint
aufgenommen wird Da in dem innenzellularen Transport
der pinocytosen Vesicula bestimmt auch Actin-Mikrofa
sern und Mikrotubula mit gleichzeitiger ATP-Benutzung
eingewickelt sind erscheinen als interessante weitere
Versuche die durch physikalische (z B Kälte) oder che
mische Mitteln (z. B Cytochalasin oder Colchicin) re
spektive die Benutzung der ATP hemmend oder Mikrofa
sern und Mikrotubula depolymerisierend weitere Proben
zugunsten des durch diese Versuche al wahr erscheinend
aktiv Transport Mechanismu der IgA bringen würden

Table I *Patients with prolonged bleeding time*

Patient	Age (years)	Ivy bleeding time (minutes)	Cause of the prolonged bleeding	Treatment
1 C S	13	19	Thrombasthenia	AMCA + fresh whole blood
E R	22	18	No definite diagnosis	AMCA
3 J H	22	15	Thrombasthenia	AMCA + fresh whole blood
4 O K	47	14	Thrombasthenia	AMCA
5 I H	32	14	Enhanced fibrinolytic activity	AMCA
6 K O	49	19	Intake of acetyl-salicylic acid	0
7 R M	52	14	Intake of acetyl-salicylic acid	0
8 P H	5	16	Intake of acetyl-salicylic acid	0
9 B R	39	16	Intake of acetyl-salicylic acid	AMCA
10 L M	40	13	No definite diagnosis	AMCA
11 M D	41	17	Unknown	0
12 M E	6.	18	Intake of acetyl-salicylic acid	AMCA
13 A L	69	16	Unknown	0
14 I L	51	14	Intake of acetyl-salicylic acid	AMCA
15 M M	57	13	Thrombasthenia	AMCA
16 A V	8	16	No definite diagnosis	AMCA
17 K P	33	18	Intake of acetyl-salicylic acid	0
18 L L	8	14	Unknown	0
19 T C	15	15	No definite diagnosis	AMCA
20 G P	20	14	No definite diagnosis	AMCA
21 M M	9	18	Intake of acetyl-salicylic acid	0
22 K N	55	18	No definite diagnosis	AMCA
23 I S	38	14	Intake of acetyl-salicylic acid	AMCA
24 J O	5	18	No definite diagnosis	AMCA
25 U L	49	18	Intake of acetyl-salicylic acid	0

Platelet count was done according to Björkman (1959) and Duke bleeding time as described by Nilsson (1974). Platelet adhesiveness according to Hellem's method (Hellem 1960). Aggregation studies of the platelets with Born's aggregation method were performed in a Payton Dual Channel Aggregation Module as described by Cronberg (1970).

Coagulation. Coagulation time in glass tubes and in plastic tubes, recalcification time of plasma and activated partial thromboplastin time, APTT (General Diagnostics) (Nilsson 1974) were studied. Factor VIII clotting activity (VIII C) was determined biologically as described by Nilsson et al. (1957), factor VIII related antigen (VIII R Ag) immunochemically in the way described by Holmberg & Nilsson (1973). Factor IX, one stage prothrombin time (P&P), factor V, fibrinogen, factor XIII (fibrin stabilizing factor) were determined by methods reported earlier (Nilsson 1974, Henriksson et al. 1979).

Fibrinolysis was tested by determination of fibrinolytic activity of plasma and resuspended euglobulin precipitate on fibrin plates, euglobulin clot lysis time and fibrin degradation products (FDP) (Nilsson 1974, Nilsson & Olow 1962).

The first Ivy test was performed on the day before operation. If the bleeding time was prolonged, surgery was usually postponed for 2–3 weeks during which time a re-test and further investigation were performed.

RESULTS

In 25 patients (8.3%) we found a prolonged bleeding time (Table I). In 10 cases the condition could be related to intake of acetyl salicylic acid since the bleeding time at re-testing after withdrawal of the drug was normal. In 3 more cases with no history of acetyl-salicylic acid intake the bleeding on repeated assay also was normalized. In the remaining

Acta Otolaryngol 89 222–226 1980

CYTOTOXIC LEUKOCYTE REACTION

E Holopainen T Palva P Stenberg A Backman
H Lehti and J Ruokonen

*From the Departments of Otolaryngology and Pediatrics
University of Helsinki Helsinki Finland*

Abstract Blood samples from 49 subjects were submitted to cytotoxic leukocyte tests which were read double blind by two laboratory technicians The results were identical in 78% of the tests and the statistical error of the method was 0 46 In a group of 32 subjects the cytotoxic test was repeated on 3 consecutive days Deviations in the results occurred in 18–70% of the tests the statistical errors varying between 0.38 and 0 43 In a series of serum samples (75 subjects) with positive leukocyte reactions to food antigens various in vitro procedures were carried out in connection with the cytotoxic test The following observations were made In 4 of 58 tests a positive leukocyte reaction was transferred with the reactive serum to cells from non-reactive subjects After heating of the reactive serum (56°C h) and washing of the cells reactivity was no longer observable in 44 out of 5? tests Treatment with EDTA consistently blocked expected cytotoxic reactions in 36 tests DSCG inhibited expected reactions in 45% antihistamine in 93% and cortisone in 70% of the tests

The cytotoxic test is based upon the observation that food antigens affect the amoebic movement and streaming of cytoplasmic granules and in stronger reactions the viability of neutrophilic leukocytes Black (1956) was the first to describe this observation and later the Bryans (1960 1971) developed a test for clinical use based on this phenomenon The mechanism underlying the cytotoxic leukocyte reaction has been rather neglected Only Black s experiments suggest that the reactivity is a property of the patient s serum Two questions have frequently been raised in the publications on the cytotoxic test the reliability of test readings and the reproducibility of the test (Lieberman et al 1975 Benson & Arkins 1976)

MATERIAL AND METHODS

1 Blood samples for cytotoxic tests w[ere] drawn in a group of 49 patients for the purp[ose] of studying the reliability of test readings T[he] testing technique was exactly the same as t[hat] reported by Bryan & Bryan (1960) Cytot[oxic] tests were carried out with 23 food antigens total of 1 127 tests and the reactions w[ere] read in a double-blind manner by two labo[ra]tory technicians

2 Another series of blood samples was c[ol]lected from 32 patients in an effort to study t[he] reproducibility of the test on consecu[tive] days The test with 23 food antigens was [re]peated on 3 successive days and the res[ults] recorded blind by the same laboratory tech[ni]cian

3 In order to analyse factors which mi[ght] influence the cytotoxic leukocyte reaction t[he] following in vitro procedures were used [in] connection with cytotoxic tests on blood sa[m]ples from 75 subjects

(*a*) The cells from non reactive subje[cts] were mixed with serum from persons w[ith] positive cytotoxic leukocyte reactions to fo[od] antigens The mixing procedure was repea[ted] in reverse order with reactive cells and no[n-]reactive serum

(*b*) Reactive serum was heated in a 56 water bath for 30 min and 2 h respectively [to] inactivate complement and IgE In this ser[ies] of tests first unwashed cells and then ce[lls] washed twice for 15 min with TRIS-BSS buf[fer]

Acta Otolaryngol 89: 479–486, 1980

ATTIC RETRACTIONS FOLLOWING SECRETORY OTITIS

M. Tos and G. Poulsen

From the University E.N.T. Clinic, Gentofte Hospital, Copenhagen, Denmark

(Received May 30, 1979)

bstract. At re-examination of 527 ears with secretory itis 3 to 8 years after tubulation otomicroscopy and mpanometry revealed different types of retraction of hrapnell's membrane in 34%; of these 4.2% had pro- unced retraction with resorption of the osseous annulus d 0.2% had atticus cholesteatoma. There was a close rrelation between the frequency and severity of retrac on on the one hand and the age of the patient at re- mination, the tympanometric conditions, and the thology of pars tensa on the other. The continued ogression is further promoted by lack of natural leaning of the retraction. As the frequency of secretory itis is very high in children the 4.2% of pronounced tractions constitute a sufficient quantitative basis for rect progression to atticus cholesteatoma.

The principal purpose of this study was to stablish the character and frequency of patho- ogical changes in the Shrapnell's membrane n well-treated secretory otitis. Another long- erm object has been to investigate whether tticus cholesteatoma is a direct after-effect of ecretory otitis or whether it develops directly rom retractions in Shrapnell's membrane.

Earlier investigations

Several investigators have studied sequelae hanges of pars tensa following secretory otitis Stevens 1962, McKinnon 1971, Kilby et al. 1972, Mawson & Fagan 1972, Kokko 1974, Bonding & Lorentzen, 1973, 1974, Tos & Poulsen, 1976) but to our knowledge there xists no systematic investigation of the hanges in Shrapnell's membrane.

Mawson & Fagan (1972) found attic retrac ions in 2% of ears after well-treated secretory otitis. Kokko (1974) found attic cholesteatoma in 0.7% of ears and McKinnon (1971) in 1.0% when including both attic and sinus cholestea- tomas.

In our oldest material comprising patients with secretory otitis treated with tubulation before 1970 and re-examined in 1975, i.e. 5 to 8 years after treatment, we found changes of pars tensa in 55% of ears (Tos & Poulsen 1976) and cholesteatoma in 1%. In addition we found different degrees of retractions of pars tensa. Unfortunately this material was not systematically investigated with reference to changes in Shrapnell's membrane. Conse- quently in 1977 and 1978 we re-examined a new material of patients operated upon from 1971 to 1975 and after systematic investiga- tions of the changes in Shrapnell's membrane these were divided into predetermined types of retraction.

MATERIAL AND METHOD

The material comprises 527 ears from children under the age of 13, the majority being be- tween 4 and 8 years of age, with secretory otitis treated under general anaesthesia with adenotomia, myringotomy and exsuction of secretion and most frequently with tubulation.

The children were most often referred from an ENT specialist and our routine method has been to insert an Armstrong tube anteriorly or anterior inferiorly in the drum. In 58 ears tubulation was not performed but only myrin- gotomy. Ten ears had much mucous secretion on this side but were not tubulated in defer- ence to the wish of the parents. 7 ears had some serous secretion and 31 ears had small or negligible amounts of mucous secretion, for which reason we estimated that tubulation was unnecessary. The patients were regularly

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Abstract Blood samples from 49 subjects were submitted to cytotoxic leukocyte tests which were read double blind by two laboratory technicians The results were identical in 78% of the tests and the statistical error of the method was 0 46 In a group of 32 subjects the cytotoxic test was repeated on 3 consecutive days. Deviations in the results occurred in 18–20% of the tests the statistical errors varying between 0.38 and 0 43 In a series of serum samples (75 subjects) with positive leukocyte reactions to food antigens various in vitro procedures were carried out in connection with the cytotoxic test The following observations were made In 42 of 58 tests a positive leukocyte reaction was transferred with the reactive serum to cells from non-reactive subjects After heating of the reactive serum (56°C 2 h) and washing of the cells reactivity was no longer observable in 44 out of 5 tests Treatment with EDTA consistently blocked expected cytotoxic reactions in 36 tests DSCG inhibited expected reactions in 45% antihistamine in 93% and cortisone in 70% of the tests

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MATERIAL AND METHODS

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2 Another series of blood samples was collected from 32 patients in an effort to study the reproducibility of the test on consecutive days The test with 23 food antigens was repeated on 3 successive days and the results recorded blind by the same laboratory technician

3 In order to analyse factors which might influence the cytotoxic leukocyte reaction the following in vitro procedures were used in connection with cytotoxic tests on blood samples from 75 subjects

(*a*) The cells from non-reactive subjects were mixed with serum from persons with positive cytotoxic leukocyte reactions to food antigens The mixing procedure was repeated in reverse order with reactive cells and non-reactive serum

(*b*) Reactive serum was heated in a 56°C water bath for 30 min and 2 *h* respectively to inactivate complement and IgE In this series of tests first unwashed cells and then cells washed twice for 15 min with TRIS BSS buffer

able VI *Relationship between pathology of pars tensa and types of retraction of Shrapnell's membrane*

rs tensa	Types of retraction 0 $N=346$ (%)	I $N=67$ (%)	II $N=91$ (%)	III $N=18$ (%)	IV $N=4$ (%)	Chole-steatoma $N=1$ (%)	Total $N=527$ (%)
ormal	57.5	26 9	26 4	5 6	–		45.2
bnormal	42 5	79 1	73 6	94.5	100.0	100 0	54.8

traction of Shrapnell's membrane Diffuse trophy with retractions of pars tensa is char cterized by a thin retracted ear drum with a iminished tympanic cavity but without ad- esion to the promontory Valsalva s test ade the drum bulge forward prominently- ith Siegle s speculum it was hypermobile etraction of Shrapnell's membrane occurred 74% of these ears. In adhesive otitis the rum adhered to the promontory was partly r entirely immobile and the middle ear was artly or entirely atelectatic There were re- ractions of Shrapnell's membrane in all ears ocalized atrophy without retraction occurred ither in the upper posterior or upper anterior uadrant or downward in tensa. Retraction f Shrapnell's membrane occurred in almost ne-third of these ears Atrophy localized pos- eriorly with retraction onto the incudostape dial joint and a pexi to the joint was accompanied in 88% of the ears by retraction of Shrapnell's membrane Diffuse tympanosclerosis or fibrosis of the drum characterized a few ears with a considerably thickened drum, in the remaining ears there was circular or horse-shoe shaped tympanosclerosis in the entire circumference of the pars tensa. Localized tympanosclerosis either posteriorly anteriorly or downwards was in 35% of cases accompanied by retraction of Shrapnell's membrane A combination of tympanosclerosis in one place and atrophy in another occurred frequently and Shrapnell's membrane was retracted in 66% of these ears

Table VII shows the clear correlation between the severity of the pathology of pars tensa and the frequency of retractions of Shrapnell's membrane in the following order

able VII *Relationship between different pathological conditions of pars tensa and types of retraction of Shrapnell's membrane*

ondition of pars tensa	No. of ears	Types of retraction 0 (%)	I (%)	II (%)	III (%)	IV (%)	Chole-steatoma (%)
iffuse atrophy	3	69	22	9			
iffuse atrophy and retraction	31	26	19	48	3		3
dhesiv otitis	13		23	38	38		
ocalized atrophy	49	69	14	16		0	
ocalized atrophy and pexi	17	[illegible]	1	47	18	12	
iffuse tympanosclerosis	20	45	35	15		5	
ocalized tympanosclerosis	79	65	14	19	3	0	
trophy and tympanosclerosis	48	44	21	21	13	2	
Total pathology	289	30 9	18.3	43.2	5.9	1.4	0 3
ormal pars tensa	238	83 6	5 9	10.1	0 4		

Table III *The effect of drugs on the cytotoxic reaction*

Drug	No of subjects	No of positive reactions in original test	Inhibited reactions n	%
DSCG	13	62	28	45
Antihistamine				
Phenergan[a]	16	44	41	93
Tavegyl[a]	16	44	41	93
Cortisone				
Betnesol[a]				
1 25000	1	31	6	19
Solu-Cortef[a]				
1 2000	1	31	7	23
1 200	12	31	16	52

the reverse mixing procedure the cytotoxic reactivity of the leukocytes remained almost undiminished in 40% of the tests despite the addition of non-reactive serum Positive leukocyte reaction was observed in 46% of tests performed with heated reactive serum (56°C for 2 h) reactive non washed cells and food antigens Incubation with food antigens produced positive reactions in 21% of tests in which heated serum was added to washed reactive cells (Table II) Similar results were obtained when the tests were carried out with serum heated for 30 min

EDTA was found to block all expected cytotoxic reactions Control cells i e non reactive cells retained their normal mobility when prepared with EDTA solutions

After addition of one drop of 5% DSCGsolution instead of sterile distilled water expected positive reactions failed to occur in 28 out of 62 testing instances (45%) (Table III) A 1% DSCG solution did not alter any of the test results The use of higher concentrations of DSCG 10 and 20% did not cause a change in the figures obtained with 5% solution

Antihistamine preparations inhibited the expected reaction in 42 out of 44 tests (93%) (Table III) Stabilization of the cell membrane occurred in some tests on samples from known reactors also when antihistamine was added 10 min after incubation with food antigen had started

Cortisone solution in a concentration 1 25000 blocked the expected cytotoxic reaction in 19% of the tests Higher concentrations (1 2000 and 1 200) inhibited the reaction in 23 and 52% respectively (Table III)

4 Histamine solution at a dilution of 1 1000 did not affect the normal cell mobility At a dilution of 1 100 some cell rounding and diminished pseudopod activity were observed When histamine chloride and histamine acid phosphate solutions were used at dilutions of 1 10 and 1 5 almost all cells were rounded

DISCUSSION

The cytotoxic reaction is apparently mainly connected with serum factors A circumstance arguing in favour of this is that the majority of reactions can be transferred with reactive serum to non reactive cells In our in vitro experiments the cytotoxic reactivity was transferred with the serum in 72% of the tests while in Black s series the corresponding figure was 100% Our observations differ slightly from the implications of Black s results also in tests performed with heated serum When the two most likely reacting serum factors complement and IgE were inactivated by heating at 56°C for 30 min and 2 h respectively we still noted positive reactions in 21% of the tests On the other hand we found that [illegible] which blocks Ca^{2+} and Mg^{2+} ions whic[illegible] sential in the classical

Table VI *Relationship between pathology of pars tensa and types of retraction of Shrapnell's membrane*

	Types of retraction						
Pars tensa	0 $N=346$ (%)	I $N=67$ (%)	II $N=91$ (%)	III $N=18$ (%)	IV $N=4$ (%)	Cholesteatoma $N=1$ (%)	Total $N=527$ (%)
Normal	57.5	26.9	26.4	5 6	–		43.2
Abnormal	42.5	79 1	73 6	94.5	100 0	100 0	54 8

retraction of Shrapnell's membrane Diffuse atrophy with retractions of pars tensa is characterized by a thin retracted ear drum with a diminished tympanic cavity but without adhesion to the promontory Valsalva's test made the drum bulge forward prominently with Siegle's speculum it was hypermobile Retraction of Shrapnell's membrane occurred in 74% of these ears. In adhesive otitis the drum adhered to the promontory was partly or entirely immobile and the middle ear was partly or entirely atelectatic There were retractions of Shrapnell's membrane in all ears Localized atrophy without retraction occurred either in the upper posterior or upper anterior quadrant or downward in tensa. Retraction of Shrapnell's membrane occurred in almost one-third of these ears Atrophy localized posteriorly with retraction onto the incudostapedial joint and a pexi to the joint was accompanied in 88% of the ears by retraction of Shrapnell's membrane Diffuse tympanosclerosis or fibrosis of the drum characterized a few ears with a considerably thickened drum In the remaining ears there was circular or horse-shoe shaped tympanosclerosis in the entire circumference of the pars tensa. Localized tympanosclerosis either posteriorly anteriorly or downwards was in 35% of cases accompanied by retraction of Shrapnell's membrane A combination of tympanosclerosis in one place and atrophy in another occurred frequently and Shrapnell's membrane was retracted in 66% of these ears

Table VII shows the clear correlation between the severity of the pathology of pars tensa and the frequency of retractions of Shrapnell's membrane in the following order

Table VII *Relationship between different pathological conditions of pars tensa and types of retraction of Shrapnell's membrane*

		Types of retraction					
Condition of pars tensa	No of ears	0 (%)	I (%)	II (%)	III (%)	IV (%)	Cholesteatoma (%)
Diffuse atrophy	32	69	22	9			
Diffuse atrophy and retraction	31	26	19	48	3		3
Adhesive otitis	13		23	38	38		
Localized atrophy	49	69	14	16		0	
Localized atrophy and pexy	1	1	12	47	18	12	
Diffuse tympanosclerosis	20	45	35	15		5	
Localized tympanosclerosis	79	65	14	19	3	0	
Atrophy and tympanosclerosis	48	44	1	21	13	2	
Total pathology	289	50 9	18.3	23 2	5.9	1 4	0.3
Normal pars tensa	238	83 6	5.9	10.1	0.4	–	

Table III *The effect of drugs on the cytotoxic reaction*

Drug	No of subjects	No of positive reactions in original test	Inhibited reactions	
			n	%
DSCG	13	62	28	45
Anthistamine				
Phenergan®	16	44	41	93
Tavegyl®	16	44	41	93
Cortisone				
Betnesol®				
1 25 000	12	31	6	19
Solu-Cortef®				
1 2000	1	31	7	23
1 200	1	31	16	5

the reverse mixing procedure the cytotoxic reactivity of the leukocytes remained almost undiminished in 40% of the tests despite the addition of non-reactive serum Positive leukocyte reaction was observed in 46% of tests performed with heated reactive serum (56°C for 2 h) reactive non washed cells and food antigens Incubation with food antigens produced positive reactions in 21% of tests in which heated serum was added to washed reactive cells (Table II) Similar results were obtained when the tests were carried out with serum heated for 30 min

EDTA was found to block all expected cytotoxic reactions Control cells i e non reactive cells retained their normal mobility when prepared with EDTA solutions

After addition of one drop of 5% DSCGsolution instead of sterile distilled water expected positive reactions failed to occur in 28 out of 62 testing instances (45%) (Table III) A 1% DSCG solution did not alter any of the test results The use of higher concentrations of DSCG 10 and 20% did not cause a change in the figures obtained with 5% solution

Antihistamine preparations inhibited the expected reaction in 42 out of 44 tests (93%) (Table III) Stabilization of the cell membrane occurred in some tests on samples from known reactors also when antihistamine was added 10 min after incubation with food antigen had started

Cortisone solution in a concentration of 1 25 000 blocked the expected cytotoxic reaction in 19% of the tests Higher concentrations (1 2 000 and 1 200) inhibited the reaction in 23 and 52% respectively (Table III)

4 Histamine solution at a dilution of 1 1000 did not affect the normal cell mobility At a dilution of 1 100 some cell rounding and diminished pseudopod activity were observed When histamine chloride and histamine acid phosphate solutions were used at dilutions of 1 10 and 1 5 almost all cells were rounded

DISCUSSION

The cytotoxic reaction is apparently mainly connected with serum factors A circumstance arguing in favour of this is that the majority of reactions can be transferred with reactive serum to non reactive cells In our in vitro experiments the cytotoxic reactivity was transferred with the serum in 72% of the tests while in Black s series the corresponding figure was 100% Our observations differ slightly from the implications of Black s results also in tests performed with heated serum When the two most likely reacting serum factors complement and IgE were inactivated by heating at 56°C for 30 min and 2 h respectively we still noted positive reactions in 21% of the tests On the other hand we found that EDTA which blocks Ca^{2+} and Mg^{2+} ions which are essential in the classical complement pathway consistently eliminated expected cytotoxic re

CERVICO-OCULAR REFLEX IN THE NORMAL ADULT

David Barlow and William Freedman

From Temple University Department of Rehabilitation Medicine Rehabilitation Engineering C nter Moss Rehabilitation Hospital Philadelphia USA

(Received April 4 1979)

Abstract (1) The cervico-ocular reflex (COR) in humans is measured hile the subjects (=10) stood on ro-tatable platform in dark room with the head fixed by stationary biteplate Eye movements were measured in response to active and passive rotations about vertical axis (2) The COR gain (I horizontal eye movement/ amplitude of body rotation) was as great as 22% at low frequency of body rotation (0 025 Hz) With increasing frequency (g., at 0 4 Hz) the gain decreased to about 7 (3) The phase angle of the eye movement ranged generally between −80 and 240 degrees with an average response around 180 degrees (4) During active rotation the COR response was similar to the responses measured during passive body rotation (5) The principal conclusion dra is that in normal adult humans the COR does not aid in stabilization of the image on the retina during passive or active body rotations. A theoretical function for the COR is presented and discussed

Early this century Bárány (1906) reported that he could observe reflexive eye movements in rabbits by fixing the rabbit s head and rotating its trunk about various axes He concluded that the reflex was tonic since the eyes re mained in their deviated position as long as neck torsion was maintained Bikeles & Ruttin (1915) were the first to report similar reflexive eye movements in man. They described hav ing produced compensatory eye movements in an 18-year-old male with inexcitable laby rinths by rotating the man s trunk while hold-ing his head fixed Bárány (1918) disclosed that he could produce similar eye movements in new borns and premature infants These re flexive eye movements could only be observed during the first two days after birth after which time they became obscured by spon-taneous voluntary eye movements

The non-vestibular origin of this reflex was recognized quite early but it was not until thirty years later that the receptor sites were convincingly shown. The strongest evidence (Biemond & deJong 1969 Hikosaka & Maeda, 1973 McCouch et al 1951) suggests that the receptors involved with the cervico-ocular reflex (COR) reside in the interverte bral joints and ligaments of the first three cer vical vertebrae

Although cervically induced eye movements were commonly seen in experimental animals and could be observed in infants and in adults under certain pathological conditions they were never seen in the normal adult (DeKleyn & Stenvers 1941) After considerable debate over whether the reflex actually existed in nor mal adults its presence was finally demon-strated when researchers began testing sub-jects in the absence of visual feedback (Philips-zoon 1962 Philipszoon & Bos 1963 Suzuki 1972, Takahashi & Oyster 1974) Visual feed-back was commonly eliminated by placing the subject in a dark room or having him close his eyes

Fixation of the head together with the re moval of visual input allows isolation of the COR from the two other primary oculomotor reflexes—the vestibulo-ocular reflex (VOR) which responds to head acceleration (Melvill Jones & Milsum, 1965) and the opto-kinetic reflex (OKR) which responds to retinal image slip (Takahashi & Oyster 1974) It has been presumed that the COR acts together with the VOR and OKR to stabilize images on the retina during active head movements (Dich-

DISCUSSION

Huljing to Holopainen You investigated various aspects of the cytotoxic leucocyte reactions Could you tell us in which kind of patients your investigations have been carried out and could you also inform us of the results of this test in normals?

Holopainen (Reply) Most patients with positive reactions in the cytotoxic test had nasal problems with perennial symptoms So-called normal subjects (controls) had negative reactions or only one-two slight reactions.

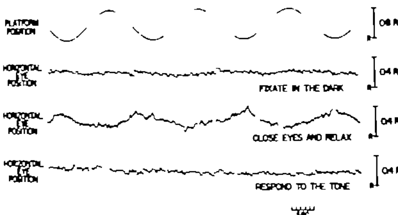


Fig 3 Comparison between COR responses produced under three sets of instructions Data shown are from one subject. Note that eye calibration (0 4 radians) is one-half that for the platform position

movements have certain repeatable characteristics

Fig. 1 contains a portion of the COR recorded from 3 different subjects The data were selected to show the general character of neck induced eye movements As illustrated in the figure during motionless periods (left side of figure) the subjects exhibited seemingly random eye movements around some central point. Upon the commencement of neck torsion, the baseline began to fluctuate These fluctuations in the baseline will be referred to slow phase eye movements (in opposition the fast phase eye movements or saccades seen in nystagmus) Since typical values for gain and phase angle are given in the following section it is sufficient at this point to make two observations first that the magnitude of the slow phase eye movements are significantly less than the magnitude of the neck movement and second that the slow phase eye movement generally occurs in the direction opposite to the direction of platform (or shoulder) movement That is a shoulder rotation to the right (clockwise rotation when observing the subject from above) results in a small eye rotation to the left (counter-clockwise when observing the subject from above)

As demonstrated in Fig. 1 slow phase eye movements appear to sum directly with spontaneous eye movements which occur in the absence of neck torsion. The random eye movements which are present before rotation begins continue on throughout the period of neck rotation

Effects of stimulus frequency

At the lower frequencies of rotation employed cervically induced eye movements are most apparent. As the frequency of neck torsion is increased the magnitude of the resulting eye movement decreases until at 0.2 Hz there is little eye movement which can be correlated with the neck torsion stimulus

The decline in response magnitude with increasing frequency is presented in Fig. 2. This figure contains the results of the computer analysis for all 10 subjects under each of the conditions used during the passive experiments. COR response gain is less than 3% at frequencies above 0 1 Hz for the Respond to the Tone and Fixate in the Dark instructions The largest response is seen at the lowest frequency examined 0 025 Hz. At this frequency the maximum mean gain observed was approximately 22%

The phase of the COR response generally ranged between −80° to −240° as seen in Fig. 2 There appears to be a slight increase in phase lag with increasing frequency of rotation although average phase angle response ranges around −180° The significance of this fact to ocular stability will be presented in the discussion

DISCUSSION

Huizing to Holopainen You investigated various aspects of the cytotoxic leucocyte reactions Could you tell us in which kind of patients your investigations have been carried out and could you also inform us of the results of this test in normals?

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rotation An assumption has been made here that neck receptors will be stimulated equally well by a body rotation (head fixed) as by a head rotation (body fixed) However the direction of rotation would be opposite That is head rotation to the right (body fixed) would be equivalent to a body rotation to the left (head fixed) Therefore an eye movement which would aid in eye stabilization (as does the VOR) would be in the same direction as the body movement—a phase angle of 0° between the eye and body movements In general then, since eye movement and body movement were 180° out of phase rather than reducing retinal image slip the COR response enhanced it. A similar finding was reported by Gresty (1976) working with rabbits

What then is the function of the cervico-ocular reflex A speculative answer to this question is suggested as follows The vestibolo-ocular reflex provides an image of head acceleration to the oculomotor centers to help in eye fixation during head movement It is proposed that the COR provides an image of body movement to the oculomotor centers The need for separate head image and body image to provide eye fixation becomes apparent during complex movements of head and body For example assume that the head is moving to the left at 5°/sec and the body is also moving to the left but with a velocity of 20°/sec In this case the VOR would produce an eye movement toward the right (responding to the 5°/sec head rate) the COR would sense the relative neck torsion to the left (15°/sec rate) and would produce an eye movement also to the right The combined eye movement produced by VOR and COR would therefore tend to reduce retinal image slip

Despite the fact that the summation of VOR and COR effects will not always occur as it does in the example the concept of an eye movement opposed to head motion (VOR) and an eye movement opposed to body movement (COR) is satisfying. Although such a function for the COR is speculative it suggests the path to experimental validation.

ACKNOWLEDGMENTS

The authors would like to thank Ruth Christaldi for her help in the preparation of the manuscript This research was supported by Grant Number 23-P 55518 from the Rehabilitation Services Administration, Department of Health, Education and Welfare Washington, D C

ZUSAMMENFASSUNG

1 Der cervico-okuläre Reflex (COR) beim Menschen wurde gemessen, während die Versuchspersonen (Zahl 10) auf einer Drehplattform standen Der Kopf der Versuchsperson wurde von einer stationären Beißplatte fest gehalten. Die Augenbewegungen wurden als Reaktion der aktiven und passiven Körperrotation um einer vertikalen Achse gemessen

2. Der Verstärkungsfaktor des COR (d h. horizontale Augenbewegung/Schwingungsbreite der Körperrotation) war so stark als 22% bei einer schwachen Frequenz der Körperrotation (0,025 Hz). Bei zunehmender Frequenz (z B 0 4 Hz) nahm der Verstärkungsfaktor bis zu % ab

3 Der Phasenwinkel der Augenbewegung lag in den meisten Fällen zwischen 80 Grad und − 40 Grad mit einer durchschnittlichen Reaktion von ungefähr −180 Grad

4 Während der aktiven Rotation war der COR den Reaktionen der passiven Körperrotation ähnlich

5 Das Hauptergebnis ist folgendes Beim normalen wachsenen Menschen unterstützt nicht der COR die Stabilisierung von dem Erscheinungsbild auf der Netzhaut während der passiven oder aktiven Körperrotationen. Eine theoretische Funktion des COR wird vorgelegt und diskutiert.

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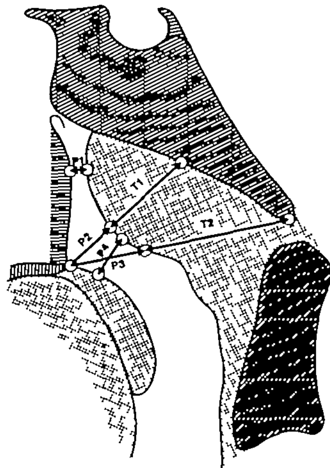


Fig 1 Radiographic measures of the nasopharyngeal airway

adenoid size and adenoidectomy is generally performed according to clinical evaluation of the children A history of nasal obstruction and snoring is the most common indication for surgical intervention but Hibbert & Whitehouse (1978) state that preoperative signs and symptoms are poor predictors of the adenoid weight and that radiography is a more accurate method as a basis for surgery

The purpose of the present study was to evaluate the relationships between (1) radiography (2) rhinomanometry and (3) clinical symptoms and signs in adenoid children in order to reach a more accurate assessment of the nasopharyngeal airway in each patient

MATERIAL AND METHOD

Twenty four children aged 4 7–12 5 years mean age 8 7 years admitted to hospital for adenoidectomy were studied They all subsequently underwent adenoidectomy and were controlled 2 months later

A history of *symptoms* was taken and especially the following symptoms were recorded (1) Recurrent Upper Airway Infection (RUAI) (2) Snoring (3) Rhinolalia (4) Mouthbreathing.

All children underwent a clinical examination by an otologist involving anterior and posterior rhinoscopy as well as inspection of ears nose and throat. No child suffered from upper airway infection at the time of examination

Radiography

Radiological examination was performed immediately after the clinical evaluation Lateral as well as postero-anterior radiographs were taken using a Lumex cephalostat In the lateral radiographs the central ray passed through the two earrods of the cephalostat which had been inserted in the external ear canals The head was thereby positioned so that the median plane was parallel to the film With the focus median plane distance of 180 cm and the film-median plane distance of 10 cm the enlargement of the lateral films was 5 6%

The following measurements were made on the lateral radiographs (Fig 1):

1 Size of the airway

P_1 The shortest distance from the most anterior part of the adenoidal mass to the posterior wall of the maxillary anthrum (Hibbert & Whitehouse 1978)

P_2 The distance from the pterygomaxillary point (the intersection of the contour of the nasal floor with the posterior contour of the maxilla) to the adenoid tissue along the line from the pterygomaxillary point to the mid point of a line joining basion (the most posterior inferior point on the clivus of the occipital bone) and the center of sella turcica (Linder Aronson & Henrikson 1973)

P_3 The distance from the pterygomaxillary point to the posterior pharyngeal wall along the line from the pterygomaxillary point to basion (Linder Aronson & Henrikson 1973)

Table II *Relationships between VS of caloric nystagmus and lesion site*

Lesion site	Loss of VS	VS
IO		
rostral	0	4
caudal	0	5
SC	7	2

abnormality of OKN OKN-DP to the left alone falls in the lower right corner (shaded area) and OKN DP to the right alone in the upper left corner (shaded area) The other three areas signify bilateral OKN diminution (lower left square) and unidirectional diminution of OK responses combined with OKN DP The lowest and highest nystagmus beats were 33 and 153 which are indicated by horizontal and vertical dotted lines respectively (Fig. 1) Thus the normal range fell in the hexagonal area After the follow-up studies were completed the brain of the animal was perfused with 10% formol–saline solution through the left cardiac ventricle The extent

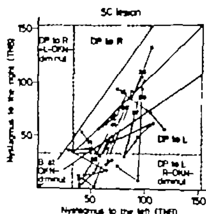


Fig 3 Optokinogram using TNB as parameter OKN in cats with SC lesions tends to show OKN-DP to the left with diminished OK responses to the right except for 2 cats (14 15) belonging to Group I

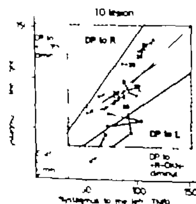


Fig 2 Optokinogram using TNB as parameter in the IO-lesioned cats The first open circle in the hexagonal area indicates TNB before IO lesions The subsequent changes of OKN according to time course are indicated with arrow which are interrupted with the cat numbers About week interval between the one end of an arrow and the other end for an open circle or square and filled circle The OKN in all the IO-lesioned cat are unaffected and every open square falls within the normal range except for cat no. 35

of lesions was determined by serial sections of paraffin-embedded specimens and reproduced by a projecting device

RESULTS

Of 42 cats investigated 30 survived the electrolytic lesions of the IO or the SC In 25 of the 30 cats, the control test could be performed thoroughly before and after lesions were made In 7 of 25 cats the lesions were found outside the target area. The data for those 7 cats were therefore discarded ultimately Table II summarizes the relationships between VS of caloric nystagmus and the lesion site VS of caloric nystagmus was revealed in all the IO-lesioned cats With the SC lesion VS was not recognized in 7 of 9 cats OKN investigated on these cats is described according to lesion site

IO lesion

In 4 of 9 cats lesions of the IO were found to cover the rostral part, but not the caudal part of the medial accessory olive (MAO) and in the remaining 5 cats lesions completely covered the dorsal cap of the MAO OKN in the IO-lesioned cats were plotted in the optokinogram A normal range of TNB in 9 cats fell in

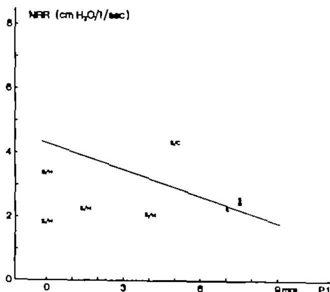


Fig 2 Association between nasal respiratory resistance (NRR) and nasopharyngeal airway at P (r=−0.52, $p<0.05$) Letters indicate main symptoms. S=Snoring H=Hearing loss C=RUAI

sue measured at T_1 was also correlated with NRR ($r=0.58$ $p<0.01$ Fig 3) whereas the correlation between T_2 and NRR was not significant ($r=0.37$)

The relationships between the symptoms are shown in Table III Probably significant correlations ($p<0.05$) were found between RUAI and Rhinolalia and between Snoring and Mouthbreathing

The relationship between the radiographic and rhinomanometric recordings and the symptoms are given in Table IV All symptoms showed correlations of about 0 3 with NRR but these were not significant with the present sample size The nasopharyngeal airway measured by the radiography was negatively correlated to the symptom Snoring ($p<0.05$ at P_1 and P_4) This is in agreement with the findings of Hibbert & Tweedie (1977) Mouthbreathing showed negative but non-significant correlations with P_1–P_4

The depth of soft tissue measured at T and T_2 showed no significant correlation to any symptom but the highest correlations ($r=0.4$) appeared with the symptom Snoring (Table V)

Measurement of the width of the nasal airway on the posteroanterior radiographs showed no correlations with radiographic rhinomanometric figures

The semiquantitative evaluation of amount of surgically removed adenoid tis showed no relationship to any other para ter

DISCUSSION

Radiographic assessment of the na pharyngeal airway can be performed w great accuracy and reproducibility when c ried out under well-defined conditions a cephalostatic fixation of the head

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By means of the above techniques the pr ent study aimed to examine in detail t relationships between radiographic a rhinomanometric measures of the nas pharyngeal airway and some clinical sym toms of airway inadequacy

The highest correlation between the radi

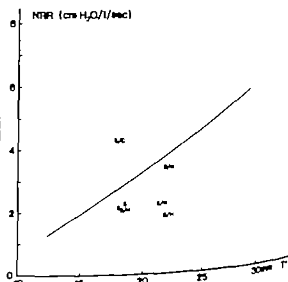


Fig 3 Association between nasal respiratory resistance (NRR) and adenoidal prominence at T (r=0 58 p<0 01) Letters indicate main symptoms. S=Snoring H Hearing loss, C=RUAI

urde an 42 Katzen OKN nach Läsion der unteren Olive und des Colliculus superior wodurch der Flocculus das visuelle Signal empfängt, untersucht. Nach Läsion der unteren Olive wurde die visuell ausgelöste Suppression des kalorischen Nystagmus immer noch beobachtet und OKN abgesehen von einer Katze auch normal ausgelöst. Nach Colliculusläsion wurde der Effekt des Lichts auf den kalorischen Nystagmus an 7 von 9 Katzen nicht beobachtet und OKN in diesen Katzen zeigte das Richtungsüberwiegen nach links und die Verminderung oder den Ausfall nach rechts. Von diesem Resultat darf man daher wohl sagen, daß Colliculus superior als praflocculärer Kern eine wichtige Rolle spielt.

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Dr I Kato
Department of Otolaryngology
Yamagata University School of Medicine
Zao-Iida
990-23 Yamagata City
Japan

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Radiographic assessment of the nasopharyngeal airway can be performed with great accuracy and reproducibility when carried out under well-defined conditions and cephalostatic fixation of the head

Recording of nasal respiratory resistance NRR has previously been reported possible in only 50% to 75% of the cases, but is now possible in all subjects down to about 5 years of age by means of a specially developed technique involving individual lining of the mask and bio-feedback monitoring of the recordings (Solow & Greve 1980)

By means of the above techniques the present study aimed to examine in detail the relationships between radiographic and rhinomanometric measures of the nasopharyngeal airway and some clinical symptoms of airway inadequacy

The highest correlation between the radio-

Fig 3 Association between nasal respiratory resistance (NRR) and adenoidal prominence at T ($r=0.58$ $p<0.01$) Letters indicate main symptoms S=Snoring H=Hearing loss, C=RUAI

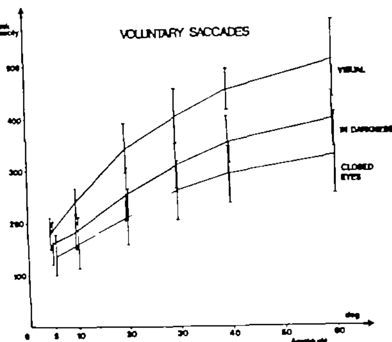


Fig. 3. Mean peak velocities with standard deviations of voluntary saccades when performed in visual conditions, in darkness and in darkness behind closed eyelids at different amplitudes.

At 20° amplitudes the mean peak velocity of the saccades in light was about 340°/s, in darkness about 250°/s and behind closed eyes about 210°/s (Fig. 3). From these values the peak velocities increased with increasing amplitudes of the saccades and at amplitudes of 60° were 500°, 400° and 320°/s respectively (Fig. 3).

Saccades were characterized by highly variable velocities when performed without visual control. Sometimes efforts to produce saccadic eye movements with closed eyes resulted in recordings appearing more as slow deviations of the eyes than as fast saccadic eye movements (Fig. 4). In darkness, overshooting saccades were very common, especially at the amplitudes of 10° and 20°.

There was further a considerable interindividual variation in saccadic velocity. Since this study was focussed on the mean values of a whole group of subjects examined in standardized visual conditions, no further interest was paid to either inter- or intraindividual variations.

We found no difference in the peak velocities of saccades between different age groups (Fig. 5). Young subjects had no higher saccadic velocity than older ones; in fact a subject aged 72 had faster saccades than another subject aged 22.

The rapid return eye movements following the pursuit eye movements were analyzed in five subjects. These rapid eye movements had the same amplitude-velocity relationship as visual saccades. Hence we considered these movements identical to the saccadic ones.

Fast phases of nystagmus. We found no difference in the peak velocities of the fast phases of caloric nystagmus, whether induced by irrigation of the right or of the left ear, by cold or by warm water. These peak velocities were, as were the velocities of the saccades, strongly dependent upon the amplitude of nystagmus as shown in Fig. 6. Moreover, the fast phases of nystagmus were equal, whether caused by rotation or by caloric stimulation. The peak velocities of the fast phases of nystagmus induced by rotation at amplitudes of 20° were about

caused by excessive adenoidal tissue and that cephalometric radiography and rhinomanometry may provide valuable information for the clinical diagnosis of this condition

ZUSAMMENFASSUNG

An 74 Kindern die an unsere Abteilung zur „Adenoidectomie" gewiesen wurden nahm man auf Grund von subjektiven Symptomen Radiograhpie und Rhinomanometrie eine Beurteilung der nasopharyngealen Luftwege vor Schnarchen war das einzige wesentliche Symptom das mit der Einengung des Nasopharynx in Verbindung gesetzt werden konnte Die nasale Atmungsresistenz stellte man in Relation zu den radiographischen Messungen der Luftwege und den Tiefenmessungen der Weichteile der hinteren Pharynxwand welche mittels einer neuen Technik gemessen wurde Dadurch konnten auch Kinder bis zu einem Alter von nur 5 Jahren rhinomanometrisch beurteilt werden Sowohl die Radiographie als auch die Rhinometrie sind wertvolle Parameter für die Indikation zur „Adenoidektomie"

ACKNOWLEDGEMENTS

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Henning Sørensen MD
University ENT-Dept
Hvidovre Hospital
DK 2650 Hvidovre Denmark

DISCUSSION

Tonndorf to Sørensen Question What breathing rate did you use

You showed us that there was no correlation between tissue mass and the breathing impairment The most prominent factor with an adenoid mass is the Bernoulle effect i e the sucking in of the soft wall which is a function of flow velocity I would therefore propose that you increase the breathing rate until flow resistance increases You will then have another useful measure

Sørensen (Reply)

In the examination we wanted the children to breathe in their natural way in order not to force either the rate or the pressure In this way we obtained the best reproducibility of the recordings in each child

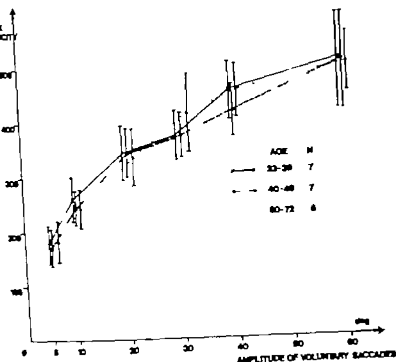


Fig. 5. Mean peak velocities with standard deviations of visual voluntary saccades in three different age groups at different amplitudes.

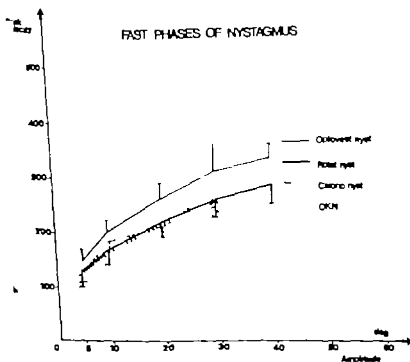


Fig. 6. Mean peak velocities and standard deviations of fast phases of optovestibular nystagmus (—), of rotatory nystagmus (—) of caloric nystagmus ( ) and of optokinetic nystagmus ( ). The spreads are presented only in direction

caused by excessive adenoidal tissue and that cephalometric radiography and rhinomanometry may provide valuable information for the clinical diagnosis of this condition

ZUSAMMENFASSUNG

An 24 Kindern die an unsere Abteilung zur „Adenoidectomie" gewiesen wurden nahm man auf Grund von subjektiven Symptomen Radiographie und Rhinomanometrie eine Beurteilung der nasopharyngealen Luftwege vor Schnarchen war das einzige wesentliche Symptom, das mit der Einengung des Nasopharynx in Verbindung gesetzt werden konnte Die nasale Atmungsresistenz stellte man in Relation zu den radiographischen Messungen der Luftwege und den Tiefenmessungen der Weichteile der hinteren Pharynxwand welche mittels einer neuen Technik gemessen wurde Dadurch konnten auch Kinder bis zu einem Alter von nur 5 Jahren rhinomanometrisch beurteilt werden Sowohl die Radiographie als auch die Rhinometrie sind wertvolle Parameter fur die Indikation zur „Adenoidektomie

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You showed us that there was no correlation between tissue mass and the breathing impairment The most prominent factor with an adenoid mass is the Bernoulli effect i.e the sucking in of the soft wall which is a function of flow velocity I would therefore propose that you increase the breathing rate until flow resistance increases. You will then have another useful measure

Sørensen (Reply)

In the examination we wanted the children to breathe in their natural way in order not to force either the rate or the pressure In this way we obtained the best reproducibility of the recordings in each child

ported the idea that all types of rapid eye movements should be equally rapid. Our observations to the contrary may have some support in previous literature also.

Thus the normal contraction of the antagonistic muscles present only during the fast phase of nystagmus (Shimazu 1972) might explain a difference in velocity between fast phases and saccades. Further, the "slow" saccadic velocities noticed e.g. in Huntington's chorea, has been believed to be due to a lack of proper inhibition of antagonistic muscles (Starr 1967).

One other factor which seems to be of importance is that saccades are initiated voluntarily but fast phases of nystagmus are released automatically and secondarily to slow phases. During voluntary saccades the subject is alert and active performing a task while during fast phases of nystagmus the subject is passive. The higher velocity of the saccades might therefore at least in part be explained by a more powerful excitation of pontine neurons caused by volition or related to alertness caused by this volition.

ZUSAMMENFASSUNG

Willkürliche schnelle Augenbewegungen (Saccaden) und reflexbedingte schnelle Augenbewegungen (schnelle Nystagmusphasen) wurden bei 20 gesunden Personen untersucht und miteinander verglichen. Die Geschwindigkeiten beider Augenbewegungstypen stiegen mit einer Zunahme der Amplituden an. E wurde gefunden, daß die willkürlichen Augenbewegungen, die Saccaden, im Licht am schnellsten, langsamer in Dunkelheit und am langsamsten hinter geschlossenen Augenlidern und. Während die Geschwindigkeiten der schnellen Phasen von optokinetischen und vestibulären Nystagmen identisch waren, erreichten die schnellen Phasen von optovestibulären Nystagmen (sowohl optische als auch vestibuläre Stimulation) signifikant höhere Geschwindigkeiten als beide anderen Typen. Bei gleicher Amplitude und unter gleichen visuellen Bedingungen waren die Geschwindigkeiten der willkürlichen schnellen Augenbewegungen (die Saccaden) signifikant höher als die schnellen Komponenten reflexbedingter Augenbewegungen.

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Fig 1 Cholesterol granuloma—the fluorescent (i e the bright spotty areas) denotes the presence of lactoferrin

H One biopsy from a tympanosclerotic region

All biopsies were also examined for control with fluorescent IgA but without antilactoferrin

RESULTS

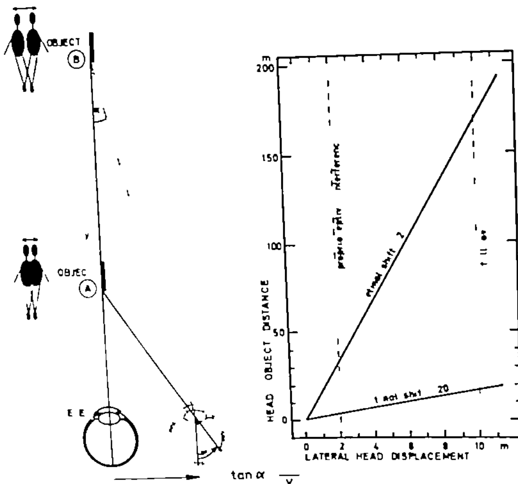
1 The 10 mucus samples were strongly positive showing fluorescent anti IgG binding to anti lactoferrin denoting the presence of lactoferrin
2 Biopsies from normal mucosa showed a faintly positive fluorescent reaction in one and hardly any reaction in the two others
3 The 17 biopsies from the dry mucosa of simple chronic otitis media were positive but not strongly so
4 All 15 biopsies from cholesterol granulomas (Fig 1) the 45 biopsies from simple wet chronic otitis media—and the 20 biopsies from cholesteatomatous ears—were strongly positive This was also the case with the salivary glands
5 All controls with fluorescent anti IgG in the *absence* of anti-lactoferrin washed away and gave negative results
6 All controls of tissues without lactoferrin i e i muscle connective tissue were fluorescet negative

In symmary (A) All secreting mucosas were positive for lactoferrin The more infected the mucosa (the more mucus it contains) the stronger the fluorescent reaction denoting a higher content of lactoferrin (B) The mastoid areas (which contain fewer mucus secreting elements) were less positive than the middle ear proper (C) All controls were negative.

DISCUSSION

A blue (steel blue) tympanic membrane can hide behind it a true post traumatic haemotympanum a bulging jugular bulb or a chemodactoma But the most common of all is the "Idiopathic haemotympanum called The Blue Eardrum which appears in prolonged secretory otitis media its colour has probably little if anything to do with the presence of the few red blood corpuscles found on microscopic examination behind the tympanic membrane—in spite of its name Fresh blood or microscopic amounts of blood are not found in these tympanic cavities

The Blue Drum is characterized by a long standing and chronic history of S O M a fact which has already been recognized by Paparella & Lim (1967) and Sheehy et al (1969) This we would like to confirm as we have seen ears which started as banal S O M turn blue after 6–10 years—and even less



g 1 Geometrical analysis indicates that in order to be ually detected, body sway must increase with increas- g distance between the eyes and the nearest stationary contrasts Angular displacements α on the retina, used by lateral head displacement, are smaller the reater the distance to the object. Diagram shows rela- tionship between head-object distance y and lateral head displacement, for given retinal displacement thresholds of either 2 or 20 minutes of arc. However because postu- ral regulation involves multiloop control, additional pro- prioceptive cues may alter sway amplitudes as well.

955) a normal lateral head sway of 2 cm would be subthreshold at a distance of about 3 m. This should lead to a perceptual conflict as the vestibular and somatosensory receptors sense a body shift which the visual system cannot detect The conflict might be resolved by increasing the postural sway and thereby reactivating visual control For a simple loop control a relationship between distance and sway amplitude could be expected (tan α = /y) with the gain dependent on the retinal movement detection threshold However with the lower limbs close together at free stance one falls over at a head sway more than 10 cm Thus at an eye–object distance of 15–20 m a maximal body sway' would produce retinal angular deviations (α) less than 20 min of arc according to the trigonometrical model. Since we are dealing with a multiloop control of postural balance it can be assumed that with increasing sway amplitudes the particular sensory weight of the somatosensory and vestibular afferences becomes greater Increasing their contribution to pos-



Fig 3 Higher magnification of a cholesterol granuloma stained with Prussian Blue—dark spots are positive stain for iron. *Arrow*—cholesterol crystals

so successful in producing cholesterol granulomas experimentally including iron deposits in mastoid cells of squirrel monkeys by obstructing the Eustachian tube for 6 to 12 months Their micrographs show black areas which they interpret as the presence of a hemorrhage

The findings of all these experiments reaffirmed that when a cavity which usually contains air (in this case the ear) is chronically underaerated an inflammatory granuloma with deposits of cholesterol crystals will appear The cholesterol crystals are either synthesized *in situ* or transported from elsewhere and deposited there The biological significance of the appearance of cholesterol granulomas is not clear The classical notion that cholesterol crystals are witness to a previous haemorrhage needs proof however This notion might be a carryover from days gone by which was not substantiated in the first place

Chronically underaerated middle ear clefts pass through episodes of variable negative pressure it is this negative pressure which is postulated as bringing about blood vessel lesions extravasation of blood which will show as a blue drum and lead to hemosiderin deposits This is in a sense an extension of the classical ex vacuo theory However the negative pressure in the clinical cases is in all probability not abrupt enough to cause rupture of blood vessels as the case is when the Eustachian tube is clamped shut in experimental occlusions There is also usually no histological evidence of bleeding blood vessels associated with such an event in ears with cholesterol granulomas It might therefore be more realistic to regard the red blood cells found in the vicinity of cholesterol granulomas as the consequence of surgery and look for another explanation for the presence of iron deposits An alternative explanation to the presence of iron deposits can be found in our detection of lactoferrin which is an iron-chelating agent We have found lactoferrin to be present in all ears where cholesterol granulomas appear—and none in controls. The explanation for the presence of cholesterol crystals eludes us so far—synthesis in situ due to the change environment is one possibility

Lactoferrin was originally detected in milk—hence its name (So & Sorensen 1939). Later lactoferrin was found to be present in all exocrine secretory organs (Massod

Actually already in 1971 was cholesterol described by Van de Calseyde et al (Acta Otolaryng 71 153–158 1971) in mucus from secretory otitis media and this could well be the source

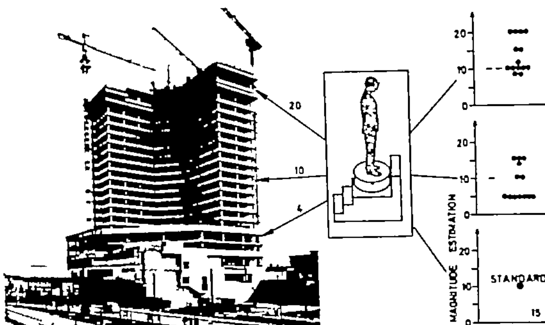


Fig. 5. Saturation of subjective height vertigo at increasing altitudes (20, 50 and 100 metres: 4th, 10th, and 20th floor respectively). Magnitude estimations of 15 subjects are not significantly different for the three altitudes. The fourth floor (20 metres) served as the standard situation (modulus with an arbitrary value of 10).

desired perceptual cues provided by windows and visible ceilings were prevented by restriction of peripheral vision to a total field of 120 degrees in diameter. Subjects were blindfolded and taken in an outside elevator to the fourth floor at an altitude of approximately 20 m. Thereupon via a staircase they stepped onto the stabilometer platform which stood adjacent to the outer wall of the building. The blindfolds were removed and the subjects suddenly exposed to the height vertigo inducing situation during which they were required to look down for a period of one minute. A security belt was used as a precautionary measure. The fourth floor condition served as the standard situation for further magnitude estimations of subjective vertigo (modulus with an arbitrary value of 10). Posturographic measurements were performed of the fore–aft and lateral body sway with eyes closed and eyes open as well as obtaining the subject's scalings of height vertigo severity. All S's were then taken from the fourth to the twentieth and finally to the tenth floor, representing an altitude of about 100 and 50 m, respectively. Technical factors within the building site prevented randomization of the order of exposure to the different heights.

As depicted in Fig. 5, statistically there was no significant difference among the height vertigo intensities experienced at altitudes of 20, 50 or 100 m, although some S's gave greater estimations on the twentieth floor. For most subjects vertigo was already maximal on the fourth floor. Thus, subjective height vertigo seems to be saturated and maximal at a height of about 20 m. Habituation may have contaminated the data, however. This is suggested by the slight decrease of the mean scaling value at floor 10 compared with the standard at floor 4.

The stabilograms of postural sway taken under these natural environmental conditions were affected by occasional wind and an extensive body tremor due to acrophobia (Fig. 6). This tremor had a frequency of 5–10 Hz and did not represent the visually induced sway of height vertigo because it continued

That lactoferrin is present in excess in middle ear granulomas could be taken as the mucosa s means of self-defence Why middle ear under aeration by itself should promote the local appearance (synthesis) of lactoferrin or indeed of cholesterol crystals is not known

ACKNOWLEDGEMENT

This work was supported in part by a NIH grant no NS-10048-08

ZUSAMMENFASSUNG

Direkte manometrische Messungen des Gasdruckes im Mittelohr wurden durch ein in der tympanischen Membrane eigens mit hohler Nadel gemachtes Loch gasverlustlos durchgeführt. Die so gemessenen jedoch kleine negative Drücke (ein paar mm von H_2O) im Falle von S O M und Atelektase sind aber falsch da ein sehr grosser bis jetzt unerachteter Korrekturfaktor angewendet werden muss Für diese Korrektur muss man aber das Volumen des freien Gasraumes des bestimmen Mittelohres genau kennen Obwohl dises Volumen im Einzelfall uns unbekannt war ist es gut bestätigt dass das Mittelohrvolumen in diesen Fällen zwischen 0,5 und ,5 ml liegt Eine mittlere Grösse von 1,2 ml wurde darum angenommen und für alle Korrekturen verwendet Unsere so korrigierten Resultate waren -34 ± 86 mm von H_2O fur 4 Fälle von S O M und $-9+86$ mm von H_2O fur 32 Fälle von Atelektase Diese Messungen wie schon solche von anderen, zeigen eine sehr breite Streuung Dafür kann mann zwei Gründe angeben (I) Dass mit einem Mittelwerte und nicht mit dem genauen Mittelohrvolumen des bestimmten Ohres gerechnet wurde (II) Dass wirkliche Schwankungen der Mittelohrdurchlüftung und deshalb des Druckes vorliegen. Der Luftungsmangel im Ohre ist deshalb eine funktionelle Störung und keine Blockung des Kanals des Eustachius

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tigo does not occur on the ground despite the fact that visual targets may be at a great distance because cues from nearby stationary contrasts (provided by peripheral vision) prevent the instigating sensory mismatch

This might become different if the ground is not aligned to the earth horizontal although the relative influence of the angle of incline/decline has not been studied thoroughly In preliminary experiments we found (with great intra- and inter-subject variability) that for a given altitude subjective height vertigo can be induced at an angle of decline of 45–50 degrees and that it progressively increases with increasing slope up to a maximal vertigo With increasing slope peripheral vision provides less information about nearby stationary contours. According to posturographic measurements the development of subjective height vertigo also requires an exposure time of 5 to 30 sec to be saturated.

All visual stimulus characteristics which have an influence on the occurrence and intensity of physiological height vertigo concomitantly affect postural balance Purkinje (1820) was the first to attribute height vertigo symptoms to a postural imbalance of upright station (he stressed the unusual and therefore unadapted stimulation at gaze downward with the stationary visual reference in an imaginary space below foot support) as later was assumed more concretely by Lee & Lishmann (1975) Brandt (1976) Bles et al (1978) and Brandt et al (1978) Purkinje (1820) and Kobrak (1974) erroneously concluded as have modern textbooks that height vertigo mainly represented a disorder in neurasthenic or psychiatric patients

Pogány s (1958) finding, that patients with vestibular dysfunction exhibited a greater susceptibility to height vertigo is now understandable since the erroneous visual signal should have a disproportionately greater sensorial weight when associated with a vestibular lesion This is analogous to our experiments (paper II Bles et al 1979) where induced somatosensory or vestibular systems disturbances (e g. when standing on foam rubber or being carried in a quivering open elevator attached to the outside wall of a building under construction) enhanced subjective height vertigo symptoms

Physical prevention of height vertigo

Knowledge of the stimulus characteristics required for the optimal visual contribution to stabilized postural balance also dictates practical advice related to the reduction of height vertigo in susceptible subjects

1 One should avoid the free upright stance in critical situations at high altitudes. This is done intuitively when grasping for stationary framework or leaning against a wall for support

2. *When looking down, one should obtain* stationary cues from nearby contrasts in the peripheral visual field (Visual stabilization of posture is served primarily by the retinal periphery while the central retina mainly serves egocentric recognition and pursuit of objects) Staring at moving objects such as clouds increases the danger of falling because additional postural destabilization through linear vection may be induced One should avoid long exposure times as height vertigo usually has a latency taking several seconds to develop Looking through binoculars is very dangerous because it restricts the visual field and introduces the unusual and therefore unadapted magnification factor

3 Body and head position should be adjusted to the gravitational *vector because* vision will receive a relatively greater sensorial weight (which is undesirable) if the otoliths are displaced beyond their optimal working range by extreme head tilt. It may also be true on the basis of other observations that the feet should be *firmly* planted on an earth horizontal surface

Physiological height vertigo versus acrophobia

Physiological height vertigo must be differentiated from neurotic acrophobia or height

Acta Otolaryngol 89 240–248 1980

EXPERIMENTAL AGEUSIA

M Ciges and J Morales

From the Department of Otorhinolaryngology Faculty of Medicine
University of Granada Granada Spain

Abstract By administering acetyl-homocysteine and D-Penicillamine to the guinea pig ageusia and hipogeusia respectively were obtained Copper concentration was estimated in serum saliva, liver salivary glands, muscle heart and tongue A significant decrease in the concentration of this metal in the liver was observed and a slight increase in the rest (average values) The results were similar for both drugs We believe that copper does not play any role in the taste process The fact that this and other metals restore taste would indicate that those metals bind the SH groups of the drugs and that they are probably responsible for the ageusia.

It is known from clinical experience that the administration of thiols reduces taste acuity This has been observed by us in some patients suffering from chronic pharyngitis and to whom acetyl-homocysteine was administered On the other hand patients treated with D-Penicillamine frequently complain of decreased taste sensitivity Henkin et al (1967) were the first to observe ageusia in different conditions treated with this drug

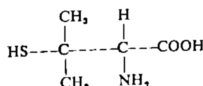
Considering that D-Penicillamine is a copper-chelating agent which depletes c it was assumed that this metal was cru taste sensitivity it was thought th ageusia observed in those cases was att ble to the lack of copper D-Penicill however is also a thiol contributor by of its SH groups as also is homocystei

Clinical and experimental experien shown that taste acuity returns to a level following the administration of co individuals treated with these drugs (He al 1969) However other transition such as zinc and nickel achieved th effect

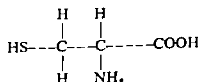
D-Penicillamine and Acetyl-homoc are drugs with similar chemical stru both are amino acids and both cont groups (Scheme 1)

It is possible to explain the action Penicillamine upon taste sensitivity

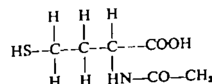
D-Penicillamine ($\beta\beta$ dimethylcysteine)



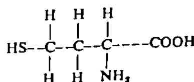
Cysteine



Acetyl homocysteine



Homo-cysteine



Schem

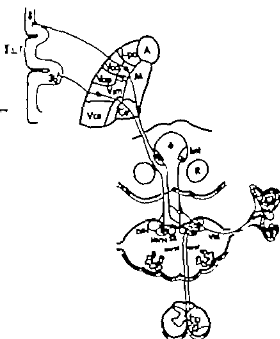


Fig. 1. Diagram showing the main ascending and descending vestibular efferents. The denomination of the ...ic nuclei corresponds to Hassler's nomenclature (LVN and MVN lateral and medial vestibular nuclei, mvst and lvst, medial and lateral vestibulospinal tracts)

...nth to the different parts of the vestibular ...uclear complex From Lorente de Nó s observations (1938) we know that semicircular ...ucts project chiefly to the superior and medial nuclei, whereas the utricle supplies the lateral nucleus (or Deiters nucleus) (Fig. 1).

The specific stimulus for the semicircular canals consists in angular acceleration or deceleration of the head which activates the ampullar cristae during movements of the head The result of this is the nystagmic reaction characterized by slow conjugated deviation of the eyes in the plane of the canal and counter to the direction of movement (slow phase) with a rapid return to the previous position (fast phase) Thus the semicircular canals that Brodal calls kinetic labyrinth records angular movements of the head This is in agreement with the anatomical data, since the medial vestibular nucleus that receives the impulses from the ampullar cristae projects in turn to the oculomotor nuclei (via the fasciculus longitudinalis medialis) and to the cervical and upper thoracic spinal cord (via the medial vestibulo-spinal tract)

On the other hand the specific stimulus for the hair cells of the utricular macula is the pressure of the otoliths over the particular region where they rest according to the varying attitudes of the head The utricule—and probably the saccule too—constitute the static or tonic labyrinth Its impulses are transmitted mainly to the lateral vestibular nucleus of Deiters and thence to the lumbar and sacral spinal cord (via the lateral vestibulo-spinal tract)

The lateral vestibulo-spinal tract terminates entirely according to histological studies of Nyberg-Hansen in the ipsilateral laminae VII and VIII of Rexed of the lumbosacral spinal cord Laminae VII and VIII contain exclusively the soma of interneuron that of motoneuron being located in lamina IX However the dendrites of the latter extend to far distant points in laminae VII and VIII (Ramon y Cajal L. de Nó Scheibel & Scheibel) There is thus histological data suggesting monosynaptic axodendritic connections between vestibulo-spinal fibres and motoneurons as well as axo-somatic contact with interneurons Since the work of Lund & Pompeiano in 1965 various authors have provided further physiological evidence in support of this view From these studies it appears that the descending lateral vestibulo-spinal fibre ends monosynaptically in α-motoneurons (Grillner et al Shapovalov Sherrington) γ-motoneurons (Grillner et al Carli et al. Pompeiano et al.) and interneurons (Grillner et al.) Grillner in 1972 succeeded in provoking monosynaptic EPSPs in ipsilateral α and γ-motoneurons of extensor nucleus of the ankle following stimulation of Deiters nucleus Only occasionally can monosynaptic EPSPs be recorded in ipsilateral extensor of the knee Indirect evidence suggests that their effects are exerted almost exclusively on static γ-motoneurons (S Grillner) Disynaptic

those to which copper is more or less tightly bound Ceruloplasmine is the best known but actually many others exist in various tissues and organs In view of these facts we investigated copper in sensory tongue mucosa saliva and salivary gland since they constitute the organ of taste and the fluid and organs having a close relationship to sense of taste

A light microscope study of the taste buds complemented this part

Second An ultrastructural study of the ageusic taste buds in order to demonstrate what was the level at which these substances actually act This part will be the subject of a second article because in most cases we used the tongues of our series for copper determination purposes

MATERIAL AND METHOD

Sixty five guinea pigs divided into three groups were studied Two groups of twenty five animals each were treated with Acetylhomocysteine and D-Penicillamine respectively until ageusia or hypogeusia appeared A third group of 15 animals was used as a control The weight of the animals was between 150 and 300 g

Five animals of each group were used for macroscopic and light microscope study The remainder were used for copper determinations

Blood serum muscle (maseter) salivary gland (submandibular) heart and liver were obtained at necropsy as well as tongue mucosa As regards the removing of the tongue and the obtaining of the saliva sample we have to be a little more explicit In so far as the tongues are concerned we knew previously the exact location of the papillae in guinea pig They have two large foliated and 20 to 30 fungiform ones widespread along the base of the tongue By using osmium tetroxide they appear dense black so we easily ascertained which was the part of the tongue that we have to remove in the future without any staining When the animals were killed by decapitation the tongue was removed and we peeled off the mucous membrane containing the papillae using a stereoscopic microscope

Saliva was obtained by suction with a pipette previously washing the mouth with distilled water and rejecting the first drops Sometimes we instilled some drops of lemon juice in order to stimulate the secretion but in most cases this was not necessary

At the beginning of the experiment we examined the taste in each animal as follows. The animal was kept for 2 days without drinking then we offered them five discs with water each with one of the four qualities dissolved in it (saccharose sodium chloride citric acid and quinine clorh) and the fifth one containing fresh water The animals immediately selected the correct disc to drink. This method was also used when testing the animals submitted to the drugs However after some experience we came to the conclusion that it was sufficient to use only one tastant because the hypogeuisa was always for the four qualities We chose as a standard substance test 0 035 quinine clorhydrate However in many cases it was necessary to use the complete test

200 milligrams per kg and day of Acetylhomocysteine in a single intraperitoneal dose was administered to the animals of the first group and to the second 28 mg/kg of D-Penicillamine by the same method

Taste was examined following the method described every 7 days and the animals were killed when ageusia was reached this generally occurred between day 20 and day 30 However in the D-Penicillamine group we could never obtain an ageusic state but only light hypogeusia and even then not in all the cases. When we increased the dosage most of the animals dies and the same happened when we prolonged the administration time for more than a month Consequently we decided to kill them as soon as hypogeusia appeared In these cases we examined the taste using a series of solutions with decreasing concentration

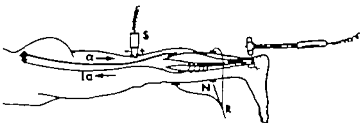


Fig 3 The position of the recording and stimulating electrodes as well as the pathw ys involve in the myotatic reflex are represented

ankle joint is passively relaxed this mini- m degree of γ-static innervation due to the ce of gravity will further diminish and the ponse thus obtained (which we call AI g. 4) will be proportionately reduced. In a mal individual the ratio AI/AR indicates proportion of γ-static versus γ-dynamic ervation whereas the index AI/M will in- ate the proportion of γ-static versus the al pool of motoneurons An increase or crease in LVST activity will respectively in ease or decrease the value of AI/AR and /M.

The Jendrassik manoeuvre has been shown provoke an increase in facilitatory supra- gmentary influences primarily of γ-dynamic d static motoneurons (J Paillard) AI and R are effectively increased during the ma- oeuvre in normal subjects The degree of gmentation is inversely proportional to the egree of excitability of motoneurons Thus in pastic patients in which the loop is already aturated the effectiveness of the Jendrassik manoeuvre to increase AI—and still more the AR—is reduced or even abolished Inversely desaturation of the γ loop by a lack of activity of facilitatory influences will relatively in crease the effectiveness

The excitability of α-motoneurons is meas- ured by the index H/M and by the recovery curve of the H reflex (J W Magladery) Passive relaxation of the ankle joint or the Jendrassik manoeuvre induces very little if any modification of the value of H and did not modify the duration of the refractory periods of motoneurons at all (N Poirier et al) In- crease in α-motoneuron excitability is mani- fested by an increase in H/M and a shortened refractory period Conversely a reduction in the α motoneuron excitability induces a fall in H/M and a lengthening of the refractory pe riod

MATERIAL

In order to determine the function of the LVST in man we compared the values of stretch reflexes in 54 healthy persons with those of patients with hyper and hypofunction of the vestibular system The former group

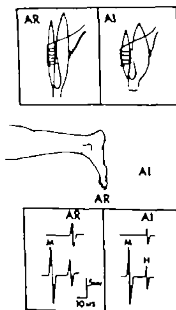


Fig 4 With the gastrocnemius in completely relaxed position the mechanically induced reflex AI is smaller than the refle obtained with the foot is a natural resting position called AR The H reflex and the M response have the same value in both instances

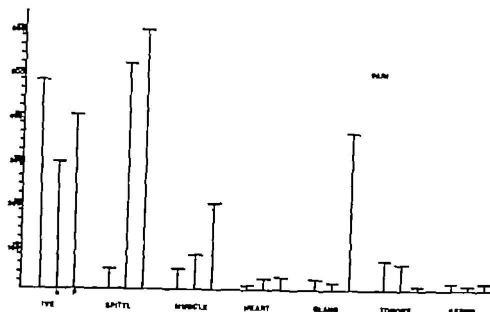


Fig 3 Variance of the copper values found. norm k. homocysteine; p penicillamine.

RESULTS

In normal animals the values obtained were

serum	9.52 γ/ml
saliva	16 42 γ/g
liver	56 80 γ/g
muscle	11 84 γ/g
heart	10 46 γ/g
gland	10 37 γ/g
tongue	10 46 γ/g

It is evident that the copper concentration in the liver is much higher than that in the remaining organs and fluids

In the organs studied and in serum concentrations were similar but a little higher in saliva (Fig 1) As we can see in Fig. 2 the dispersion index is low and the same is found with the variance values except in the liver (Fig 3)

All the animals treated with Acetyl homocysteine could not distinguish fresh water from quinine clorhydrate solution between 20 and 30 days giving the same result in the cases in which we used other tastants We therefore came to the conclusion that taste was eliminated and the animals were killed

In so far as the D-Penicillamine treated animals are concerned the results were different because we could not obtain ageusia in any case We lost many animals by increasing the dosage or prolonging the time It was necessary to include more animals to maintain the required number We examined these animals on the 25th day of treatment by using a battery of different concentrations in order to detect slight hypogeusias Only in 17 cases did we obtain it for all four qualities The rest were excluded Copper was also investigated and the values obtained were practically the same as in the hypogeusic animals

In the animals treated with Acetyl homocysteine in which we obtained ageusia, the results were

serum	12.03 γ/ml
saliva	26 96 γ/g
liver	28 76 γ/g
muscle	13 49 γ/g
heart	10.54 γ/g
gland	8 98 γ/g
tongue	9 84 γ/g

The liver copper concentration fell in relation to normal animals from 56 80 to 28 76 γ/g (Fig 1) a significant decrease As we can see in Figs 2 and 3 there is an evident discrepancy between the figures of variance and dispersion

In saliva the amount is clearly increased (from 16 42 to 26 96) and slightly so in serum

Table II. Modification of ipsilateral stretch reflexes following caloric (0°) stimulation (1 case)

	H reflex	H/M	AI	AR	AI/AR	AI+J	AR+J	AI/H	AI/M	AR/H	AR/M
[illegible]	5	31% M. 16 mVs		5	40%	4 (+100%)	8 (+60%)	40%	12%	100%	31%
[illegible]	6	37%	4	8	50%	5 (+25%)	8 (0)	66%	25%	133%	50%
[illegible]	20%	6%	+100%	+60%	+19%	−75%	−100%	+26%	+13%	+33%	+25%

roup 2. Temporary vestibular hyperfunction ring caloric (0°) stimulation

his experiment was performed in a single se of benign positional vertigo. With the tient lying on his back, the stretch reflexes ere provoked with mechanical and electrical mulation. A few seconds after commencing rigation a marked increase in AI and a oderate increase in AR were detected (Table I). The effect was bilateral but more pronounced ipsilaterally. Since the values of AI d AR are increased the effectiveness of the ndrassik's manoeuvre is proportionally reuced. Caloric activation of the labyrinth ould therefore seem to enhance the excitability of γ-static and to a lesser degree γ-dynamic motoneurons.

Group 3. Modification of stretch reflexes ollowing surgical lesion of the estibular nerve

Eighteen patients with various vestibular pathologies were selected in whom pre and post-operative studies of myotatic reflexes were performed. The results (Table III) correspond exclusively to ipsilateral reflexes obtained within 2 weeks after surgery. From the clinical standpoint all patients presented a marked abnormal Romberg test that persisted although progressively attenuated for 1 to 18 months. But even after 2 years with the patient's feet placed in front of one another and the eyes closed the Romberg test is still abnormal when the foot on the side of the neurectomy is behind the other.

Immediately after surgery there is a marked fall in the value of AI and a much more moderate decrease in AR and H (Table III). Since Jendrassik's manoeuvre is still strongly positive for AI after surgery it appears that the suppression of vestibular function provokes a selective diminution of the excitability of γ-static motoneurons. Repeated studies after periods of up to 5 years following surgery

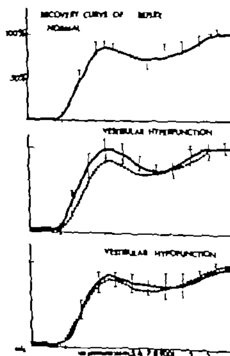


Fig. 5. Recovery curve of the H reflex in normal persons and in patients with hyper and hypofunction of the vestibular system. The dotted lines in the second and third curve correspond to the average normal result.

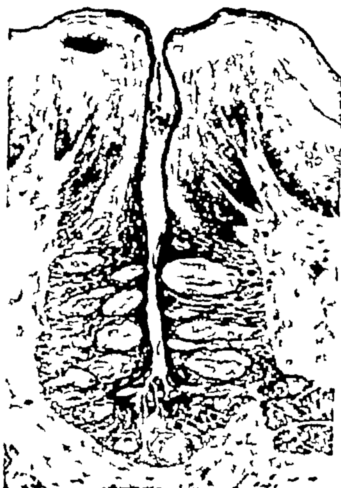


Fig 6 Enlargement of Fig. 5

In serum a slight decrease was obtained (from 9 52 to 8 44) and the same was found in the tongue (from 10 46 to 8 60) As for the rest slight increases were observed (Fig 1) The dispersion index was increased except in the tongue (Fig 2) and the variance gave no homogeneous values (Fig 3)

No macroscopic or microscopic alteration in the tongues / could be detected (Figs 4 5 and 6) However in some papillae we could observe a plug of amorphous substance in the entrance to the papillary groove and a lack of amorphous substance in the buds

DISCUSSION

It is obvious that Acetyl homocysteine provokes ageusia in guinea pigs but the results are different as far as D-Penicillamine is con cerned since we could only obtain slight hypogeusias in some cases and always bo line with toxic effects

Considering our results as a whole we that the previous works in which only co in serum was studied consider the pro only partially as copper metabolism is plex and is carried out mainly in the l Hence according to our results in serum would have to say that D-Penicillamine duces copper whereas Acetyl-homocys does not This was actually the conclusio previous works However when we com the results on the liver we see that it is at level at which an important decrease place in both cases

In the rest of the organs and fluids stu copper levels were slightly increased. The erages were

Acetyl homocysteine from 11.56 γ per ml to 13 63

D-Penicillamine from 11.56 γ per g or m 13 35

It seems that these drugs deplete co from the liver at least in the time and at dosage used The copper homeostasis is m tained primarily and almost exclusively biliary excretion (Sass-Kortsak & Beam 1 and it would have been very interesting estimate copper concentrations in bile as w but we overlooked this aspect of the probl It is possible that copper follows two pathw from the liver serum and organs and excre via bile Thus we may not expect low value serum until later periods of intoxication

In the tongue saliva and salivary gla there is no significant quantity of copper think of a direct action of this metal in the ta process On the other hand from the action the drugs copper is not decreased as we sa and the result with similar values of metal co centration was different because with o drug we produced ageusia but not with other Hence we arrived at the conclusi that copper does not play any part in ta process Our figures in the dispersion stat tics however are higher in general terms the animals treated than in the controls Th

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M Ciges M.D
Dept of Otorhinolaryngology
Faculty of Medicine
Granada
Spain

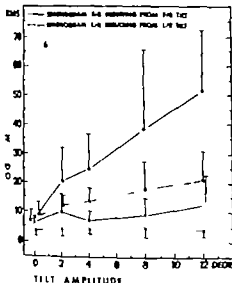


Fig 4 Mean RMS values (arbitrary units, ± 1σ) of lateral and fore-aft stabilograms Both the lateral and the fore aft stabilograms are obtained from lateral resp fore-aft sinusoidal tilt of Tilting Room II (freq 1/40 Hz, ampl. 2, 4 8 and 12°) The 6 subjects were standing both without ( ) and with (O) foamrubber on top of the stabilometer

attached to the head and restricting the visual field to 75° For each condition six stabilograms were recorded, no one or two layers of foamrubber with or without a funnel It must be emphasized that under these natural conditions it was not possible to control all stimulus parameters of the visual scene or to avoid occasional presence of a slight breeze

As can be seen in Fig. 3 the instability is already maximal at an eye-object distance of 25 m. No significant difference between the RMS values for the different test conditions obtained at 25 75 and 200 m could be established This is also the case when peripheral vision is present. However when using foamrubber the levels of instability (RMS values) obtained with peripheral vision are below the levels found with the restricted visual field ($p<0.01$)

The RMS of the stabilograms obtained in the eyes-closed' conditions is of the same order of magnitude as the RMS obtained with eye-object distances of at least 25 m without peripheral vision Ranking the subjects according to the RMS in both the balcony and tilting room experiments gives a coefficient of rank correlation of 0 82, indicating that the role of vision in postural stabilization is subject-dependent Since the postural instability is already maximal at a distance of 25 m the data of experiment I do not sufficiently determine the saturating relationship between eye-object distance and sway amplitudes below 25 m Therefore a second experiment was designed for testing the influence of small distances as well as the differential effects of optokinetic stimulation on fore-aft and lateral body sway

Experiment II Lateral and fore-aft body sway at various distances (0.5 to 25 m) and proprioceptive interference

Six healthy subjects (4 male 2 female age 20-4?) participated in this experiment.

First they were tested in Tilting Room II both with and without foamrubber The results are shown in Fig 4 There is a remarkable discrepancy with the results of Expt I Although the RMS obtained with one layer of foamrubber in Tilting Room II is less than that found in Expt I the subjects could not perform the test on double foamrubber Most of the subjects preferred to have some assistance available even with only one layer of foamrubber because they felt unstable and sometimes experienced vertigo

It is interesting to note that the subjects were much more sensitive to the fore-aft than to the lateral tilt.

The Tilting Room II experiment was followed by a more natural stimulus situation for testing the influence of the distance below 25 m under static conditions In this experiment a university lecture hall was used to provide different eye-object distances thus eliminating the possibility of wind influencing the results By a suitable choice of illumination and the use of an adapted funnel the eye-object distance could be varied up to 25 m The distances in the lecture hall at which stabilograms were recorded were 0.5 ..5 5

Table I *Pituitary tumours—clinical and experimental material*

	Total	GH	PRL	Chromo-phobe	ACTH	Normal
No of operated cases	57	31	1	11		1
In vitro						
A	31	19	6	3		1
B	6	1	3			
Total	37	20	9	5		1

Cases operated on at the ENT Department
[b] Cases operated on at the Department of Neurosurgery

turcica The material is summarized in Tables I and II

Methods

1 *General considerations*

The principles for organ culture of pituitary tumour tissue have been described in detail by Anniko et al (1979*a*)

Between 24 and 36 pieces from individual tumours were explanted to the in vitro system and observed over a 6–30-day period of culture The culture media were analysed for both GH and PRL The nutrient solution was renewed every second day

All concentrations in the diagrams are calculated as hormone secretion during 24 hours and expressed as percentage of basal secretion i e the hormone secretion during the first 2 days in culture

2 *Incubation with bromocriptine*

The stock solution of bromocriptine had a concentration of 1 100 000 (10 ⁵ g/l 10 μg/ml) dissolved in isotonic saline solution This was diluted with culture medium to reach the c centrations 10^{-6} 10^{-7} 10^{-8} and 10^{-9} g/l, 1 0 1 0 01 and 0 001 μg/ml Incubation w bromocriptine was performed 2–5×48 h af an initial period of 2×48 h in organ cult medium only

3 *Morphological processing*

After ending the culture period in vitro sp mens were prepared for light and elect microscopy (cf Anniko et al 1979*a*)

RESULTS

I *The normal pituitary gland*

A discrepancy was evident concerning th vitro synthesis/secretion of GH and Pl respectively (Fig 1) In all but three organ tures totally 24 the hormone concentratio PRL decreased after a few days in vitro remaining three specimens showed a redu synthesis/secretion of PRL from the 6th on The GH level in the culture medium creased more rapidly than that of PRL

Table II *Pituitary tumours—type of tumour/age of the patient*

Type of tumour	No of patients/age (years) at operation						
	<20	≥20–30	≥30–40	≥40–50	≥50–60	≥60–70	≥70
GH	1	3	1	4	8	3	
PRL		7	4	1			
ACTH				1		1	
Chromophobe				4	4		1
Total	1	10	16	10	1	6	1

This patient was 13 years old

Acta Otolaryngol 89: 541–546 1980

SAC DECOMPRESSION FOR REFRACTORY LUETIC VERTIGO

Michael M Paparella Chong Sun Kim and Donald A Shea

From the Department of Otolaryngology University of Minnesota, Minneapolis USA

(Received August 16, 1978)

bstract. Luetic hydrops can occur in both congenital d acquired lues and otologic manifestations may be indistinguishable from Meniere disease Eight endolymphatic sac decompression procedures were performed on patients with refractory luetic vertigo Of those 3 had ngenital and 3 acquired lues All cases had received tensive anti-luetic medical therapy without any discernible improvement in the vertiginous attacks. In our 6 tients with luetic hydrops the main complaint was vere refractory vertigo indeed disabling in most cases. stoperatively all cases remained free from these severe tacks and the vertigo was controlled or eliminated in all ses Although hearing was improved in one patient, aring seemed not to improve generally speaking, as sult of the long-term postoperative follow-up W describe our method of sac decompression and drainage and ggest that this procedure might be considered for patients with uncontrollable luetic vertigo who have not nefited from adequate medical therapy

uetic hydrops may mimic the symptoms of leniere s disease However luetic hydrops is haracterized by bilaterality earlier onset, and apidly progressing symptoms The most disabling symptom of luetic hydrops is episodic ttacks of vertigo

Eight endolymphatic sac decompression rocedures were done on 6 patients with refractory luetic vertigo in an attempt to relieve heir recurrent vertiginous attacks which were nremitting and unresponsive to conservative reatment We also hope to prevent further earing impairment

It is our intention here to describe this problem and to recommend consideration of surgical decompression of the endolymphatic sac n patients with luetic vertigo where extensive medical management has hitherto failed

REVIEW OF THE LITERATURE

Luetic hydrops may be seen in both congenital and acquired syphilis In 1966 Karmody & Schuknecht reported that 38% of 123 congenital syphilitics had a hearing loss most of whom had associated vestibular symptoms with episodic vertigo and diminished caloric responses They demonstrated a case of endolymphatic hydrops due to syphilitic osteitis of the otic capsule Similar findings were observed by Alexander (1928) and Mayer & Fraser (1936) Perlman & Leek (1952) stated that endolymphatic hydrops secondary to invasion of the labyrinthine capsule may have some resemblance to the changes reported in Meniere s disease This may account for the occasional similarity of symptoms Schuknecht (1974) suggested that the inner ear reaction to lues is characterized by progressive endolymphatic hydrops and degeneration of the membranous labyrinth resulting in rupture of the membranes due to overaccumulation of endolymph

Pulec (1972) reported that 7% of his patients with Meniere s syndrome complex were found to have a syphilitic etiology and Shea & Bowers (1975) showed that 1 2% of the patients with a fluctuating hearing loss had a syphilitic origin. Severe episodic vertigo with nausea and vomiting in congenital syphilis is indistinguishable from Meniere s disease and it may precede hearing loss by months or years and

This research has been supported in part by USPHS 1 PO1-NS-12125

Table I *Pituitary tumours—clinical and experimental material*

	Total	GH	PRL	Chromo-phobe	ACTH	Normal
No of operated cases	57	31	12	11	2	1
In vitro						
A	31	19	6	3	2	1
B	6	1	3	2		
Total	37	20	9	5	2	1

Cases operated on at the ENT Department
[b] Cases operated on at the Department of Neurosurgery

turcica The material is summarized in Tables I and II

Methods

1 *General considerations*

The principles for organ culture of pituitary tumour tissue have been described in detail by Anniko et al (1979*a*)

Between 24 and 36 pieces from individual tumours were explanted to the in vitro system and observed over a 6–30-day period of culture The culture media were analysed for both GH and PRL. The nutrient solution was renewed every second day

All concentrations in the diagrams are calculated as hormone secretion during 24 hours and expressed as percentage of basal secretion i e the hormone secretion during the first 2 days in culture

2 *Incubation with bromocriptine*

The stock solution of bromocriptine had a concentration of 1 100000 (10^{-5} g/l 10 μg/ml) dissolved in isotonic saline solution This was diluted with culture medium to reach the centrations 10^{-6} 10^{-7} 10^{-8} and 10^{-9} g/l 1 0 1 0 01 and 0 001 μg/ml Incubation bromocriptine was performed 2–5×48 h an initial period of 2×48 h in organ cu medium only

3 *Morphological processing*

After ending the culture period in vitro s mens were prepared for light and elec microscopy (cf Anniko et al 1979*a*)

RESULTS

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Luetic hydrops may mimic the symptoms of Meniere's disease. However, luetic hydrops is characterized by bilaterality, earlier onset, and rapidly progressing symptoms. The most disabling symptom of luetic hydrops is episodic attacks of vertigo.

Eight endolymphatic sac decompression procedures were done on 6 patients with refractory luetic vertigo in an attempt to relieve their recurrent vertiginous attacks which were unremitting and unresponsive to conservative treatment. We also hope to prevent further hearing impairment.

It is our intention here to describe this problem and to recommend consideration of surgical decompression of the endolymphatic sac in patients with luetic vertigo where extensive medical management has hitherto failed.

REVIEW OF THE LITERATURE

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Pulec (1972) reported that 7% of his patients with Meniere's syndrome complex were found to have a syphilitic etiology and Shea & Bowers (1975) showed that 1.2% of the patients with a fluctuating hearing loss had a syphilitic origin. Severe episodic vertigo with nausea and vomiting in congenital syphilis is indistinguishable from Meniere's disease and it may precede hearing loss by months or years and

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Table IV *Ratio GH/PRL in GH producing human pituitary tumours in vitro*

Table illustrating three main groups of GH/PRL ratios Pituitary no 17 (specimens 19 and 20) has an extre high secretion of GH approximately 700 000 pmol/l/48 h (cf Table III) In contrast tumour no. 29 has a synthesis/secretion of GH into the culture medium (2 750 pmol/l/48 h) Both tumours have an approximately secretion of PRL but the GH/PRL ratios appear completely different. In this case the difference in the GH ratio indicates differences in the synthesis/secretion of GH However tumour no 20 has a concomitant hyp retion of GH and PRL. Both hormones are found in very high concentrations in vitro but the GH/PRL rat similar to that of tumour no 29 In conclusion the GH/PRL ratio does not always present the secretional act of the tumours

GH pmol/l PRL µg/l

Specimen no		Days in vitro 0–	3–4	5–6	7–8	9–10
17	19	3 469 1	?710 1	1 113 1	430 1	558 1
	20	1 667 1	1 813 1	1 153 1	336 1	886 1
29	15	69 1	58 1	9 1	5 1	13 1
	17	6 1	7 1	3 1	4 1	7 1
0ᵃ	18	3 1	7 1	7 1	9 1	9 1
	23	9 1	9 1	6 1	4 1	3 1

GH cum PRL prior to surgery treated with bromocriptine 3 months

It has to be noted that the in vitro behaviour of the tumour specimens could not be correlated to the tumour size or invasiveness of the sella turcica Histological sections of more than 400 specimens did not reveal any pituitary cells in mitosis

III *PRL hypersecreting tumours*

In general the PRL hypersecreting tumours adapted better than GH producing tumours to the in vitro system with regard to the synthesis/secretion of hormone A total of tumours were explanted to in vitro culture

Table V *GH tumours GH and PRL production in vitro – 1 mg w w – 24 hours*

A significant difference occurs in the secretional capacity of GH between individual tumours as illustrated by tumour no 17 (specimens 9 and 10) and no 29 (specimens 13 and 15) The former shows a decrease in the synthesis secretion of GH with increasing time in vitro The secretion of PRL increases during this time in culture The tumour with a low synthesis/secretion of GH appears more stable in vitro The secretion of GH is approximately the same during the first 8 days after explantation whereas the synthesis/secretion of PRL shows some fluctuations In conclusion a low synthesis/secretory capacity of a tumour tissue is better preserved unchanged in the artificial environment than tumour tissue having very high synthesis/secretion of hormone

Specimen no			Days in vitro 0–	3–4	5–6	7–8	9–10
H 17							
	9ᵃ	*GH*	175 000	167 500	147 900	77 500	65 000
		PRL	68	48	158	230	183
	10	*GH*	135 000	14 000	80 000	55 000	30 000
		PRL	78	34	103	130	163
H 29							
	13	*GH*	1 200	920	980	1 450	
		PRL	107	68	70	170	
	15	*GH*	1 030	1 045	1 080	990	
		PRL	150	18	319	117	

up to 3 hours at age 25. Her hearing began to deteriorate progressively in both ears starting 5 years ago It was fluctuating and progressive, worse on the right ear after a flu-like illness. She had syphilis at age 19 and was treated with Bismuth therapy She had also received a 70-day course of penicillin therapy 10 years ago

On admission (August, 1973) audiometric examination revealed a bilateral sensorineural hearing loss with an SRT of 60 dB (R) and 70 dB (L). Discrimination scores were 54% (R) and 58% (L) and SISI scores were 100% on both ears at 4 kHz. Caloric response was markedly reduced bilaterally with very slight subjective sensation in the left ear and none in the right ear VDRL was reactive and the FTA-ABS test was 4+ in blood and VDRL was negative in CSF Bicillin was given for 4 weeks with a diagnosis of late latent acquired syphilis. Subsequently additional medical therapy failed to control her symptoms and an endolymphatic sac decompression procedure was performed in September 1973 on the right ear and in February 1974 on the left ear

She is now rehabilitated Postoperatively she has had a mild sensation of pressure in her ear and slight imbalance However she has had no further vertiginous attacks and she is very happy being able to drive her car again.

DISCUSSION

Histopathologically the lesion of congenital syphilis cannot be differentiated from that of the acquired type It is characterized by progressive hydrops of the endolymphatic system, degeneration and atrophy of labyrinthine structures with rupture of the membranous labyrinth, causing severe incapacitating episodic vertigo accompanied by nausea and vomiting, fluctuating hearing loss and reduced speech discrimination. Tinnitus, loudness intolerance sensation of fullness in ears, and diplacusis are also present as in Meniere s disease Among those symptoms the most disabling symptom in luetic hydrops is refractory vertigo

Of our 6 patients, 4 cases initially presented with vertiginous episodes and 2 with cochlear symptoms (hearing impairment and tinnitus) However at the time of admission the patients chief complaint was incapacitating episodic vertigo which could not be controlled by conservative measures such as penicillin steroids erythromycin antiemetics sedatives or tranquillizers Three patients were diagnosed as late congenital syphilis with interstitial keratitis and Hutchinson s teeth (case 2) and the other 3 patients as acquired syphilis.

Intensive specific anti-luetic medical therapy may fail to prevent the onset of luetic vertigo deafness or to stabilize the disease process A hearing loss may occur despite adequate previous antibiotic or steroid therapy due to the variable response of the disease Our patients had received previous anti-luetic treatment without any significant effect in controlling incapacitating vertiginous episodes or progressing hearing impairment

In adequately treated syphilis viable spirochetes are occasionally found in eye CSF synovial fluid and lymph nodes and in the temporal bones of penicillin-treated congenital syphilitic deafness Spirochetes have been found in patients adequately treated with heavy metals Tamari & Itkin (1951) reported that despite administration of 9 million units of penicillin in 15 days, both hearing and vestibular function continued to deteriorate. The effective level of penicillin in vitreous humor spinal fluid or labyrinthine fluid against the spirochete is still open to question.

The patient who is allergic to penicillin has a serious problem in treatment South et al (1964) reported a case of a gravid woman treated with 15 g of erythromycin estolate over a 10-day period 2 months prior to delivery Her syphilitic infant died and the fetal level of erythromycin was only 1/5 to 1/20 of the maternal level Case 5 was allergic to penicillin and she was treated with erythromycin for



Fig 3 Light microscopy (*LM*) fixation of prolactinoma tissue (no 19). The tumour is richly vascul Hormone granules can be observed i cells. One group of cells (*arrow*) intracellular vesicles (degenerative especially active cells?) ×470

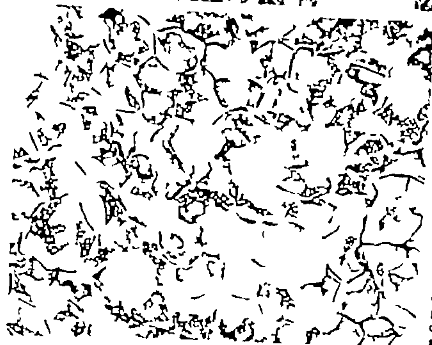


Fig 4 LM Specimen from the s tumour as in Fig. 3 In vitro culture du ×48 h (basal secretion/adaptation to or culture) but thereafter exposed to 1 g/l (0 1 μg/ml) of bromocriptine in 1 culture medium for 4 days. Severe d generation of cells ×470

degree to which neural and hormonal factors can affect hormone production (Kohler et al 1969) Also pituitary tumours may be exposed to hypothalamic control factors As observed in the present study and also earlier reported (cf Tixier Vidal & Farquhar 1975) the synthesis and release of GH and PRL differed with increasing time in vitro

The maintenance of the secretion of PRL in culture favours a predominant in vivo effect of the prolactin inhibiting factor (PIF) On the other hand the decrease in the synthesis/ secretion of GH indicates an in vivo effe of a GH-releasing factor Recently this wa also suggested by Peillon et al (1979) How ever the maintenance of the secretion by pituitary tumour cells in vitro suggests an autonomous secretion although this may be modulated in vivo by extra pituitary factors

Long-term culture of human pituitary tissue has been reported by Lipson et al (1979) de scribing hormone release but in decreasing concentrations during six months in vitro. Anniko et al (1979*b*) have reported on pituitary

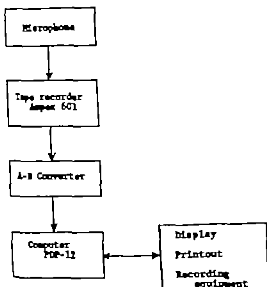


Fig 4 Block diagram of the system

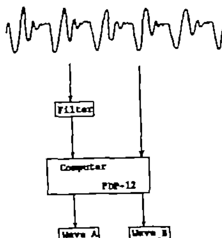


Fig 5 Voice wave digitized without filter (B) and with filter (A)

extracted It should be noted that the third harmonic on the display is actually the first harmonic of the voice waveform (Fig. 3)

METHOD

A block diagram of the system is shown in Fig. 4

1 *Voice recording*

The subjects were 14 males and 14 females with no laryngeal or pulmonary disorders and 20 males and 10 females with various laryngeal diseases resulting in various degrees of hoarseness (Table I)

A recording of each subject's voice was made in a sound-treated booth using a Electrovoice model 666 microphone and an Ampex model 601 tape recorder A stable portion of the sustained vowel /a/ was used for the analysis

Table I *Types of voice disorders*

Type of disorder	N of cases
Recurrent nerve paralysis	11 (2)
Atrophy	7 (3)
Polyp	7 (1)
Polypoid degeneration	1
Carcinoma	1
Nodule	1
Acute laryngitis	1
Mutational voice disorder	1 (1)

Numbers in the parentheses represent postoperative

The voices on the magnetic tape were edited and arranged randomly in order to avoid listener judgment bias Five persons who had experience in voice study were asked to judge the degree of hoarseness for each subject. The degree of hoarseness was rated as follows 0=none 1=slight 2=fair 3=extreme and the scores were averaged for each subject

2. *A/D conversion*

A 325 msec section of the voice signal was filtered with a 600 Hz low pass filter and digitalized (wave A) The same section of the voice signal was digitalized without filtering (wave B) (Fig 5) The sampling rate of the A/D conversion was 22026 points per second This rate was determined by the sampling program and both waves were stored on computer data tape

3 *Pitch extraction*

Wave A was displayed on a scope and three pitch periods (T) were measured using a cursor Fourier analysis was performed on the

cell morphology and hormone secretion has also been reported by Anniko et al (1979*c*) in prolactinomas exposed to bromocriptine in vitro though they found that morphological damage seldom takes place

Contradictory findings have thus been reported in the literature One explanation might be the fact that the tumour biology varies considerably among individual tumours although hypersecreting the same type of hormone thereby also giving different results following pharmacological or surgical treatment

ZUSAMMENFASSUNG

Von 56 operierten Tumoren hatten 31 eine Ubersekretion an Zuwachshormon (growth hormone = GH) 12 Tumore eine vermehrte Sekretion an Prolaktin (PRL) eine Übersekretion an ACTH und 11 Tumore waren chromophob ohne Hormonsekretion Eine Hypophyse mit erhöhter Hormonausscheidung zeigte normale Verhältnisse bei der chirurgischen Untersuchung was bei einer erneuten endokrinologischen Kontrolle bestätigt werden konnte 36 hormonausscheidende Tumore waren einer in-vitro-Kultur unterworfen worden 20 Tumore mit Akromegalie 9 Prolaktinoma Bildung von ACTH und 5 chromophobe Adenoma Je größer der Grad an klinisch nachweisbarer Akromegalie war desto höher war die Konzentration an Zuwachshormon in vitro Tumore mit einer Ubersekretion an Zuwachshormon zeigten auch eine Ausscheidung von kleinen Mengen Laktationshormon in vitro Das Verhältnis GH/PRL war für jeden Tumor individuell der eine Übersekretion an Zuwachshormon oder Prolaktin hatte Das Tumorgewebe zeigte ungefähr die gleichen charakteristischen Merkmale in vitro unabhängig davon ob das Gewebe verschiedenen Teilen des Tumors entnommen worden war Eine gute Korrelation zeigte sich bei der Sekretion von Prolaktin in vivo und in vitro d h je hoher der Serumspiegel and Prolaktin was, desto größere Konzentration an Laktationshormon wurde in den in-vitro-Kulturen gefunden Eine Mitose war in keinem der histologischen Schnitte von mehr als 400 verschiedenen Proben nachweisbar Die Zunahme an Prolaktin in In vitro-Kulturen ist wahrscheinlich abhängig von dem Fehlen eines das Prolaktin hemmenden Faktors (Prolactin-inhibiting factor = PIF) bei der in vitro-Kultur Das Absinken des Niveaus an Zuwachshormon dürfte im Gegenteil abhangig sein von dem Fehlen eines das Zuwachshormon freisetzenden Faktors Die Ergebnisse der vorliegenden Studie deuten auf eine individuelle Biologie in jedem hormonabsondernden Tumor hin

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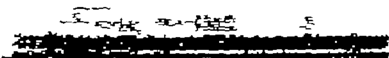


Fig 12 Sudden change in voice quality (case 9).

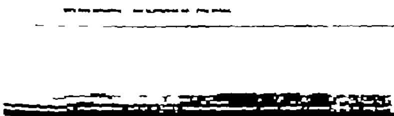


Fig 13 Sudden change in voice quality (case 13).

tion or vocal shimmer was analysed by Koike (1969), von Leden & Koike (1970) and Kitajima (1976) The previously mentioned investigators using computer analyses, have been successful in evaluating a rough quality which is related to an irregularity of the fundamental cycle

When combined with the previous methods, the calculation of the S/N ratio of hoarse voice can distinguish a breathy voice from a rough voice Furthermore the S/N ratio of hoarse voice obtained by the present method appears to be a quantitative indicator of the degree of hoarseness thereby providing objective information on the effectiveness of individual treatment.

ZUSAMMENFASSUNG

Die harmonischen Bestandteile in der heiseren Stimme wurden von den Lärm-Bestandteilen durch einen kleinen Laboratoriums-Computer abgesondert. Das Verhältnis von Harmonie zu Lärm oder H/L-Verhältnis (S/N Ratio) wurde berechnet und verglichen zu dem Gehöreindruck von 98 Personen. Die kalkulierten Resultate zeigen einen Zusammenhang zum Gehöreindruck und es wurde vorgeschlagen, Daß dieses eine hilfreiche Methode sein würde für eine quantitative Bewertung von Heiserkeit.

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Acta Otolaryngol 89 258–264 1980

BIOMECHANICS OF THE GIRAFFE LARYNX AND TRACHEA

D F N Harrison

From the Institute of Laryngology and Otology London England

Abstract Despite possession of a well developed larynx and a gregarious nature the Giraffe is able to utter only low moans or bleats Morphological and histological examination together with measurements of trachea and subglottic area on three fresh larynges (Giraffa camelopardalis) has made it possible to explain the lack of vocal power Factors such as thoracic expiratory flow rate length of trachea and recurrent laryngeal nerves together with morphological details of vocal folds and intrinsic laryngeal muscles have all been considered providing a unique example of the relationship between morphology and function in the mammalial larynx

Although the suborder Ruminanta contains many cud-chewing artiodactyles probably the most fascinating family are the Giraffidae This family is only found in Africa and includes two genera—the Giraffe and the Okapi It is accepted that giraffes are the tallest living animals and their strange appearance caused consternation when first seen in Caesar s Rome around 50 B C The Romans called it Camelopardalis for the Greeks thought it to be a mixture between camel and leopard One thousand years later the Arabs named it Zarafah (one who walks quickly) and in Italian Giraffa Its scientific name is a combination of old and new—*Giraffa Camelopardalis*[1]

Giraffes are browsers feeding on leaves and shoots at the top of acacia and other trees For this they need long necks and although having only 7 cervical vertebra can grow to a height of over 4 6 m (15 feet) with an adult weight of almost 2 030 kg (2 tons) The body is relatively short resulting in a neck which can measure almost 2.5 m (8 feet) in length For the purpose of grading size is related to height with large males being over 4 6 m females 4 m and immature animals being less than 2 5 m

Since a 1 6 m column of blood exerts a pressure nearly equal to 125 mm of mercury the arterial pressure at heart level of a standing giraffe must exceed this in order to perfuse the brain Measurements have shown that blood pressure at brain level is 215 mmHg rising to 350/250 mmHg when the head is lowered to drink Despite the physiological hypertension arteriosclerosis is rarely found at autopsy and this was substantiated in my own examination of the carotid vessels of three specimens who died in captivity

Although Giraffes are gregarious animals living in family groups or large herds relationships are loose and animals often join or leave without apparent reason Parental bonds between mother and young are weak and since suckling periods are short the immature animal may wander away from parents to join other groups or even perish Almost all field studies agree that vocal communication seems to be minimal restricted to an occasional snort or at most—a weak bleat or moan Communication seems to be visual taking advantage of their great height and large eyes Sudden panicky movement by one animal may result in a stampede of the whole herd even though no visible danger is apparent Being shy and inoffensive creatures giraffes defence against predators is primarily speed However their forefeet are powerful and it has been known for an enraged bull to crush the head of a lioness A galloping giraffe may appear ungainly and relatively slow However top speeds of over 56 km per hour (30 mph) have been recorded and this cannot be matched over distance even by a horse

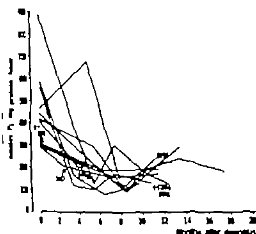


Fig 1 Eleven patients with initially increased ATPase activity In 10 cases the activity decreased to normal levels after radiotherapy Four patients died within the control period and their individual survival time (in months) indicated by figure in brackets. *ND* Neck dissection. *R* Remaining tumour mass or relapse. *M* Metastatic disease

creased lymphocyte ATPase activities Individual values are shown in Table I in which the staging of the tumours and the clinical response to treatment in these patients also appear Following radiation therapy the increased ATPase activities were normalized in all these patients (Fig. 1) except in one namely a patient who died before the first control The lymphocyte ATPase activity was normalized also in ? of the patients despite persisting tumour mass in one and bilateral lymph node metastases in the other

In 7 patients (Table II) the ATPase activity rose temporarily from an initial normal level to significantly increased values (Fig. 2) after radiotherapy This observation was not in any case related to the clinical course of the disease Only 1 of these patients died from relapse and metastases within the control period (29 months after therapy)

The remaining 27 patients (Tables III and IV) also had normal ATPase activities before radiotherapy and the activity remained normal in 19 of these patients who were followed repeatedly after treatment (Fig. 3) In no case did the lymphocyte ATPase activities seem to be correlated to the clinical course of the disease Three of the patients who had a remaining tumour mass after radiotherapy were operated on and they finally succumbed to their disease (Table III) Two other patients died from causes not related directly to their neoplastic disease

After a mean observation period of 30 months 36% of the patients had relapsed among those having an initially increased ATPase activity whereas only 15% relapsed among the patients with a normal ATPase activity before therapy However this difference is not significant ($p>0.2$ Fisher s exact test)

A clinical evaluation of the patients after

Table II *Seven patients with initially normal lymphocyte ATPase activity but in whom the activity later increased after therapy (Fig 2)*

At 3-year follow-up after therapy one patient (14%) had died of tumour relapse and metastases
TL Total laryngectomy PL Partial laryngectomy

Initials	Age (yrs)	Sex	Tumour localization	Tumour stage	Effect of treatment	Operation	Length of observation period (months)	Relapse at the end of observation	Initial ATPase activity (nmoles P_i/mg/h)
TAMN	63	♂	Glottis	$T_1N_0M_0$	Incomplete	TL	42	–	153
EOK	52	♂	Supraglot	$T_{1a}N_0M_0$	Complete	TL	29	+	153
OC	46	♂	Glottis	$T_{1a}N_0M_0$	Incomplete	PL	38	–	46
GEB	77	♂	Glottis	$T_{1a}N_0M_0$	Complete		43		198
TTL	52	♂	Glottis	$T_{1a}N_0M_0$	Complete		42	–	86
IKB	86	♂	Glottis	$T_1N_0M_0$	Complete		44	–	140
AA	65	♂	Glottis	$T_{1a}N_0M_0$	Complete		36	–	263



Fig 3 Macroscopical iew of t[h]
mencement of trachea and oeso
The right recurrent nerve lies [b]
these two structures and passes ben
lowest fibres of the inferior con
muscle The right pararecurrent ne
on the lateral surface of the oes
joining the superior laryngeal
superiorly

been made to measure the volume of the trachea directly by determining water capacity Patterson et al estimated a volume of 2 5 litres in a 16 foot (4 9 m) giraffe whilst Robin et al (1960) estimated tracheal volume by assuming the trachea to be a cylinder and applying the formula

$V = \pi r^2 l$

where V = tracheal volume in ml r is the mean radius in cm and l is the length in cm He calculated the volume in one animal as 1 6 litre In fact the trachea of the giraffe is oval in section (Fig 1) and in the largest male in my specimens (15 years old) was 51 mm widest diameter 35 mm shortest diameter with a tracheal length of 2 3 m This gives an approximate dead space volume of 3 0 litres although this does not include the volume of pharynx bronchi and bronchioles and is thus an underestimate Dead space volume in the 13 year female trachea diameter 45 mm × 28 mm was estimated as 1 8 litres Only a few giraffe lungs have been weighed and these figures have not always been correlated with the animals size or weight Figures vary from 6 to

12 kg and in my two adult specimens for
fixed lungs weighed 7 2 kg for the male
6 42 kg for the female Patterson et al ca
out in vivo studies related to lung volume
pulmonary ventilation on three giraffes
their results may not be reliable since the
mals were restrained or tranquillised H
ever it may be supposed that by compar
with man—and the giraffe is at least e
times heavier volume of dead space is
proximately nine times greater total l
capacity about eight times larger and tidal
umes range from 2 7 to 4 1 litres It appe
that the giraffe may use two mechanisms
maintenance of alveolar ventilation The e
nomical way to breathe through a long tub
to do it slowly with larg einfrequent breat
dead-space air before it can ventilate the lu
In conditions of captivity giraffes brea
about 8 to 12 times per minute In addition
tal lung capacity may be unusually large
though the merit of this is to increase inshu
tory and expiratory capacity This is of gre
importance when the giraffe is running and e
piration is probably helped by diminution
the tracheal lumen by contracture of th
muscle which is found posteriorly beneath th

Table IV *Eight patients with initially normal lymphocyte ATPase activity and in whom only one ATPase determination was carried out*

At follow-up 3 years after therapy one patient (12%) had died from tumour relapse and metastases

Patients	Age (yrs)	Sex	Tumour localization	Tumour stage	Effect of treatment	Operation	Length of observation period (months)	Relapse at the end of observation	Initial ATPase activity (nmoles P_i/mg/h)
FP	59	♂	Supraglot	$T_3N_0M_0$	Complete	–	25	–	170
AFB	64	♂	Glottis	$T_3N_0M_0$	Incomplete		10	+	105
EFD	59	♀	Glottis	$T_{1a}N_0M_0$	Complete		26	–	165
AK	54	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	26	–	138
LAL	68	♂	Glottis	$T_{1a}N_0M_0$	Complete		26	–	195
SEI	54	♂	Glottis	$T_2N_0M_0$	Complete		25	–	104
FC	65	♂	Glottis	$T_1N_0M_0$	Complete	–	28	–	169
SAL	51	♂	Glottis	$T_1N_0M_0$	Complete	–	26	–	58

Tumour cell specific sensitization of the killer cells could be due to circulating tumour cell associated antigens, but non-specific killing may also be effective (Pross & Baines, 1977). The energy needed for the cytotoxic potential, details of which are still not understood, could be provided by hydrolysis of ATP by mitochondrial Mg-ATPase. As a matter of fact it has previously been shown that a mitochondrial ATPase activity is present in human normal lymphocytes in the blood (Ellegaard & Dimitrov 1973) and that this ATPase activity is increased in patients with various neoplastic tumours including carcinoma of the larynx (Ellegaard & Dimitrov 1972*a*, 1972*b*, Dimitrov & Ellegaard 1977, Ellegaard et al 1975*a*, 1975*b*, 1975*c*). Contrary to what was believed from the beginning it seems to be the B-lymphocytes which carry the highest ATPase activity in unstimulated normal human lymphocytes isolated from the blood (Kragballe & Ellegaard 1978) and elevated ATPase activity is also found in lymphocytes from patients with B-lymphocyte proliferative diseases like chronic lymphocytic leukaemia (Ellegaard 1979).

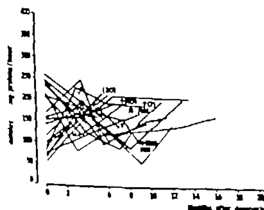


Fig 3 Nineteen patients with initially normal or subnormal ATPase activity. After radiotherapy the ATPase activity decreased in 7 patients, while it increased in the 11 other cases. Five patients died within the control period, 3 from relapse or metastases, the 2 from causes not related to their malignant disease. *PL* Partial laryngectomy. *TL* Total laryngectomy. *R* Relapse. *RM* Relapse and metastases.

The normal range for the lymphocyte ATPase activity is higher in the present study than in the previous investigation (Ellegaard et al 1975*c*). This is most likely due to alterations in the methods omitting removal of the sticky populations of cells by passing the mononuclear cell suspensions through glass-bead columns. The present study has confirmed that the ATPase activity of circulating lymphocytes is increased in a number of patients with carcinoma of the larynx and that the ATPase activity in these cases decreases to normal levels after radiotherapy. Increased protein, RNA and DNA synthesis has been demonstrated in human lymphocytes transformed



Fig 5 This is a section taken deeper to that shown in Fig. 4 and shows the large arytenoid and attached fibres of the thyroarytenoid muscle



Fig 6 Section through the right half of the larynx of an adult female giraffe This illustrates the large cartilage of Wrisberg bracing the aryepiglottic fold and the broad attachment of the thyro-arytenoid muscle to the front of the arytenoid

geal nerves of 3 human larynges He counted the total number of myelinated fibres in both the internal and recurrent laryngeal nerves finding a total of approximately 4000 with a unimodal distribution the peak lying between 8–12 μm Suggestion that these nrves are a plexus of the vagus represented by a continuous nerve joining internal and recurrent nerves and that separation from this strand forms the various laryngeal nerves" would certainly explain the numerous variants found in the mammalian larynx

Since the electrophysiological and biochemical parameters of nerve fibre function are related to fibre diameter and the longer myelinated alpha motor fibres lie in the Group I range (up to 25 μm) the number of these fibres in the recurrent nerve are of some importance when considering laryngeal function In the human fibres of 10 μm in diameter would allow a conduction velocity of about 50–65 m S^{-1} with longer refractory period than in motor nerves to the limbs In view of the considerable length of the recurrent laryngeal nerves in the giraffe they commence within the thorax as in man it is of interest to examine the number and size of the large fast conducting myelinated fibres within the nerve The technique used is based upon that described by Holland (1978) the specimen being stained with uranyl acetate and embedded in Epon Details of these findings will be described later when all six nerves have been studied for accurate measurement is tedious and time consuming. Myelinated nerves pre

chiae and ecchymoses which tend to bleed easily Localized deposits of amyloid are common in oral plaques diffuse thickenings or translucent papillas Amyloid infiltration has been reported in certain odontogenic tumors (Vickers et al 1965) The deposition of amyloid frequently precedes the calcification in the tumor

There have been only a few reports of amyloid deposits in the tonsils (Mutschler 1933 Chiari 1937 Eriksen 1970) In these, the presenting symptoms varied from no apparent disturbance to severe obstruction

The present study describes a unique case of amyloidosis of Waldeyer s ring and electron microscopic observations of large amyloid deposits and the cells surrounding them in palatine tonsils

CASE REPORT

A 35-year-old male patient born in Morocco was admitted to our department in February 1977 Six years prior to the present admission he was treated for acute maxillary sinusitis Three months before admission he had an attack of acute tonsillitis, which was treated successfully by ampicillin Five weeks later another episode of tonsillar infection occurred He was referred to our outpatient clinic because of a mild feeling of discomfort in the throat which had persisted since the last episode of infection

On exam nation both tonsils were found to be enlarged and firm Several yellowish plaques were observed in their anterior surface and an additional large plaque measuring 3 0.4 m was seen over the left posterior pillar Several yellowish masses occupied the base of the tongue and encroached on the epiglottis Another mass was seen in the left vallecula A plaque ×0.3 cm in size spread upward from the upper border of the oropharynx to the lateral wall of the nasopharynx on the left side and another small plaque was seen in the middle of the posterior wall of the nasopharynx. The patient was in good physical condition and examination revealed no other pathological findings

Laboratory findings peripheral blood hemoglobin 13 8–15 0 Gr% ESR (Westergren) 10 mm in the first hour leukocytes 5700–7700 per mm³ with a normal differential count. Urine analysis including microscopic examination of sediment disclosed no abnormality Blood levels of glucose urea uric acid albumin globulin creatinine bilirubin cholesterol electrolytes alkaline phosphatase SGO transaminase prothrombin calcium and phosphorus were all within the normal range Creatinine clearance was 125 ml/min serum folate 8 8 ng/ml serum B_{12} 266 pg/ml Serologic tests. Wassermann Kahn VDRL, cold agglutinins Rose Waaler Paul Bunnell latex, rheumatoid factor antinuclear factor monotest were all negative Anti-DNA level was 12 1% LE cells were not found Measurement of blood immunoglobulins showed IgA 168 mg% IgM 67 mg% and IgG 1830 mg%

In vitro stimulation of peripheral blood lymphocytes by lectin mitogens (phytohemagglutinin Concanavalin-A pokeweed mitogen) was normal

Tonsillectomy and biopsy of the nasopharyngeal and hypopharyngeal tissue masses were done All the studies revealed amyloidosis as determined by Congo Red staining. Biopsies of rectal and buccal mucosae did not show any signs of amyloid deposits

METHODS

Different regions from the excised right tonsil were fixed in 2.5% glutaraldehyde in cacodylate buffer pH 7 4 for 2 hours and post fixed in 1% osmium tetroxide for one hour Following dehydration in a graded ethanol series and propylene oxide they were embedded in Epon 81 (Luft 1961) Semithin sections were stained with toluidine blue ultrathin sections were stained with uranyl acetate and lead citrate

Mitwirkende Umstände wie expiratorische Ausflussgeschwindigkeit Länge der Trachea und des Nervus recurrens sowie morphologische Detaile der Stimmfalten und intrinsischen laryngealen Muskeln wurden alde in Betracht gezogen Sie gaben uns ein bemerkenswer es Beispiel der Beziehung zwischen Morphologie und Funk tion der säugetier Larynxe

RESUMÉ

Bien que la girafe (*Giraffa camelopardalis*) soit munie d'un larynx largement développé et d'une nature grégaire elle n est capable que d'émettre des bêlements et des gémissements dans le registre grave L examen morphologique et histologique accompagné de mesures de la trachée-artère et de la zone subglottique de trois larynx fraîchement prélevés a rendu possible une explication de ce déficit de force vocale Ces facteurs suivants ont été considérés la vitesse d'écoulement de l'air expiré du thorax longueurde la trachée-artère longueur de la branche récurrente du nerf pneumogastrique les détails morphologiques des cordes vocales et la structure des muscles intriseques du larynx Ces facteurs offrent un example unique de la rélation entre la morphologie et la physiologie du larynx chez les Mammifères

ACKNOWLEDGEMENTS

I wish to express my thanks to Marwell Zoological Gardens for the larynx and trachea of VICTOR and to the Zoological Society of London for the two specimens of Giraffe larynges with trachea.

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D G N Harrison M.D
The Inst of Laryngology and Otology
330/336 Gray s Inn Road
London WC1X 8EE
England

DISCUSSION

Diamant to Harrisson What happens to race-horses w have the condition called roaring ? Their efforts sudde ly diminish and they slow down while running. What is t pathogenesis?

Tonndorf to Harrison Did I understand you correc when you said the lack of tone pitch is due to the low speed? The pitch s solely determined by the tension of t vocal cords if you disregard the rare case of overblow when the pitch goes up one octave

I would suggest that the air flow is too low to set t vocal cords vibrating.

Sadé to Harrison Can the okapi the giraffe s cou speak'? It seems to have a neck no larger than the hor

Spoendlin to Harrison I wonder how the gira coughs, a function of the larynx which presumably mportant in an animal with such a long trachea.

Harrison (Reply)

Roaring in the horse is due to left recurrent nerve par and is found in the large dray animals or the genetical related inbred racehorse Pathological studies suggest t this is probably secondary to dilatation of the left atrio Ortner s Syndrome. Surgery is unsuccessful as the a mal requires a large glottis for effective activity such running

High subglottic pressure is only one of the necessa factors for good sound production. However the la dead space in the giraffe makes flow rates unusually lo Undoubtedly the long recurrent nerve and possibly sl conduction time together with simple type of larynx add to this animal's problems

Cough s not a feature of the giraffe s behaviour pos bly because of the high protective aryepiglottic folds a herbivorous diet

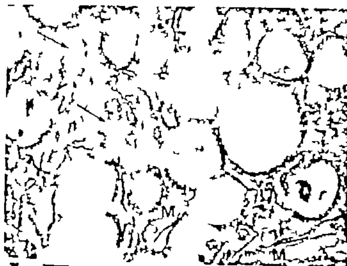






Fig. 7. Vacuoles containing varying amounts of vesicles, granular material, small dense bodies and empty regions. Note numerous profiles of RER (*arrow head*) and small vesicles (*arrow*) in the cytoplasm. *M* mitochondria. ×13 090.

Fig. 8. Clear vacuoles, some of which contain membranous and myelin-like components (*arrow*). *N* nucleus. ×12 180.

Fig. 9. Region of vacuolated cell containing numerous mitochondria (*M*) and microfilaments (*arrow*). *I* amyloid inclusions. ×33 060.

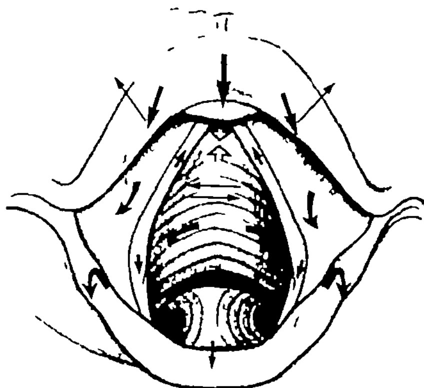


Fig 1 Lymphatic drainage of the inner larynx anterior aspect.

RESULTS

Endoscopical lymphangioscopy

The lymphatic system of the larynx is composed of two interconnected lymphatic net works i e a superficial capillary and a deeper collecting channel system The density is greatest in the area of the false cord and lowest in the posterior vocal cord Injection of patent blue into the various areas yields following results

(*a*) Injecting the laryngeal aspect of the *epiglottis* dye is drained to the free margin into the ary epiglottic fold to the false cords to the anterior commissure and penetrating the epiglottic cartilage into the base of the tongue

(*b*) From the *false cords* lymphatic drainage occurs towards the ary-epiglottic folds into the piriforme sinus and subsequently following the course of the superior laryngeal artery Dorsally there is connection to the posterior commissure the arytenoid processes and the posterior parts of the true vocal cords

(*c*) The *true vocal cord* lymphatics are mostly parallel orientated with some branching towards the ventricle At the free margin there is a sharp demarcation towards the subglottic space except for the anterior commissure where a midline crossover is possible underneath

(*d*) Injection of the *ventricle* leads to a quick drainage of dye dorsally via the arytenoid process into the false cords and anteriorly into the anterior commissure The true vocal cords and the anterior part of the false cord remain unstained

(*e*) In the *subglottic space* the lymphatics run in a circular fashion A midline crossover occurs as a rule

It has to be emphasized that during in vivo dye injections active transport of the patent blue leads to the demonstration of a primary lymphatic network and of a collecting channel system far apart from the dye depot In post mortem specimens the lymphatics are demonstrated at the periphery of the depot and the spread of the dye is—compared to the in vivo studies—limited The interstitial space is stained in a diffused manner leading to a compartmentation which is not found during in vivo studies

ACKNOWLEDGEMENTS

I gratefully acknowledge the advice and assistance of Prof. M. Pras, Sheba Medical Center and thank M. O. Pollack and Mrs G. Buckler of the Photography Department, Municipal Governmental Medical Center, Tel-Aviv.

This work was supported by the Research Committee of the Municipal Governmental Medical Center, Tel-Aviv.

ZUSAMMENFASSUNG

Amyloidosen der Mandel ist ein seltener Zustand und über Amyloidosis des Waldeyer Rings wurde bisher nicht berichtet. In der vorliegenden Studie wird ein Fall von Amyloidosen der Mandel, des Nasopharynx und der Zungenbasis bei einem 35jährigen gesunden Patienten ohne klinische Symptome beschrieben. Die Möglichkeit einer systemischen Amyloidosis wurde durch klinische Untersuchung und Biopsie von rektalen und buccalen Mucosae ausgeschlossen. Elektronenmikroskopie zeigte das Vorhandensein typischer amyloider Fibrillen. Große Mengen der Fibrillen waren von Zellen, die phagocytisch erschienen, dicht umschlossen. Die Ultrastruktur dieser Zellen wird beschrieben. Bei diesem Fall handelt es sich um einen einzigartigen Typ organbeschränkter Amyloidosis des Waldeyer Rings.

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E. Shanon, M.D.
Department of Otolaryngology
Municipal Governmental Medical Center
Ichilov Hospital
6 Weizman Street
Tel-Aviv
Israel

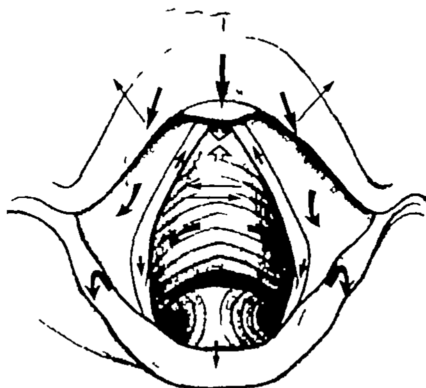


Fig 1 Lymphatic drainage of the inner larynx, anterior aspect.

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AUTHOR INDEX





Fig 3 Lymph-collecting vessel of the false cord with valve *HI* Histiocyte *VC* veil cell. Inset: Comparison of lymphatic-capillary endothelium (above) and vascular endothelium (below) *Lu* lumen *LC* Lymph capillary *BC* blood capillary *VC* veil cell

vessel level show a sharp and segmental delineation in a horizontal and vertical plane is unfeasible For the laryngeal lymphatic capillaries midline-crossing anastomoses are seen in the supra and infraglottis as well as connections with the trachea It is at the free margin of the true vocal cords where the only sharp demarcation between the capillary system of the supra and infraglottis can be detected At the collecting vessel level there are connections between supra and infraglottis between epiglottis and the base of the tongue between false cords and piriforme sinus and between infraglottis and trachea This means—in accordance with clinical observations—that there are multiple superficial and deep interconnections of the laryngeal lymphatic system The lymphatic drainage always occurs





Fig 3 Lymph-collecting vessel of the falls cord with valve *HI* Histiocyte VC veil cell. Inset: Comparison of lymphatic-capillary endothelium (above) and vascular endothelium (below) *Lu* lumen *LC* Lymph capillary *BC* blood capillary *VC* veil cell

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The second point is that the superficial supraglottic tumor rarely goes to the glottis because unless lymph drainage exists, the linea superior is the barrier for spreding of the tumour Deep infiltration of the supraglottic space proceeds very rapidly paraglottically in the second or third compartment of the larynx.

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COLLABORATORS

Austria. L. Hörbst, F Krejci, E. H. Majer O Novotny S. Unterberger
Canada. D P Bryce, J Fredrickson, W J McNally J A. Sullivan
Denmark. J Falbe-Hansen, Th. Vilstrup
Finland: H Björk, B. Grahne, U Siirala, E. Vaheri
France: M Aubry L. G Chevance, G Greiner P L. Mounier Kuhn, M. Portmann
Germany A. Herrmann, H. G Loebell, A. Miehlke, R. Mittermaier H. H. Naumann, K. H. Vosteen, H. Wullstein, F Zöllner
Great Britain. G H Bateman, I. S Hall, D F N Harrison, R. D. Owen
Greece: J Chrysikos, L. Papangelou, G E. Yannoulis
India. J V De Sa, A. B. N Rao C. Satyanarayana, P N. Sinha
Israel. J Sadé
Italy M. Arslan, E. Bocca, F Brunetti
Japan. T Daito T Fukuda, M. Goto, I. Kirikae, M. Morimoto, J Ono, S. Sato
Netherlands. L. B. W Jongkees, W H. Struben
Norway H. F Fabritius, T Leegaard, O Ophelm, S. Quist Hanssen, O Strömme
Sweden. G Dohlman, H. Engström, G Herberts, L. Holmgren, Hj. Koch, G Lidén, N Lundgren, A. Sjöberg
Switzerland: F Escher E. Lüscher A. Montandon, C. R. Pfaltz, L. Rüedi, J P Taillens, A. Weder
USA. L. F Boies, J E. Bordley T Cody D A. Hilding, H. P House, G Kelemen, J R. Lindsay M. M. Paparella, H. F Schuknecht, B. H. Senturia, G E. Shambaugh, Jr., F A. Sooy W P Work
USSR. M. Kchodiakov S. Khechinashvili, N. A. Preobrashensky

Conferences and Meetings

1980 July 9–16 Tenth International Congress on Acoustics to be held in Sydney *Australia.* Information 10th ICA Congress Secretariat, GPO Box 2609 Sydney NSW Australia 2001

1980, July 20–23 International Symposium on "Neuronal Mechanisms of Hearing" to be held in Prague, *Czechoslovakia.* Information. J Syka, Institute of Experimental Medicine, Czechoslovak Academy of Sciences, 128 08 Prague 2, U nemocnice 2, Czechoslovakia.

1980 September 2–5 IV International Symposium on Facial Nerve Surgery to be held in Los Angeles, *California,* USA. Further information address Facial Nerve Symposium, c/o Ear Research Institute, 256 South Lake Street, Los Angeles, California 90057 USA.

1980, September 21–22 The XXXI Congress of the Polish Otolaryngological Society will be *held in* Poznań. Further information. Ass Professor Antoni Pruszewicz, Clinic of Otolaryngology Academy of Medicine, 49 Przybyszewskiego Str., 50 355 Poznań, Poland.

1980, September 27–28 The Research Forum, under the joint sponsorship of the Committee for Research in Otolaryngology of the American Academy of Otolaryngology and the Association for Research in Otolaryngology will be held *in* Anaheim, California. Abstract format instructions from Professor *Makoto Igarashi,* M.D., Department of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030, USA.

1980 October The Ear Research Institute announces a two-week *Temporal Bone* Surgical Dissection Course. Information. Antonio De La Cruz, M.D., Director Temporal Bone Surgical Dissection Course, Ear Research Institute, 256 South Lake Street, Los Angeles, CA 90057 USA.

1980 October 3 A Meeting of the O R.S. (Oto-Rhino-Laryngological Research Society) will be held at the Royal National Throat, Nose and Ear Hospital, Gray's Inn Road, London. Information Professor P *Stell,* Ch.M., F R.C.S., Department of Otolaryngology Royal Liverpool Hospital, Prescot Street, Liverpool. L7 8XP England.

1981 March 22–27 Second International Conference on Cholesteatoma & Mastoid Surgery to be held in Tel Aviv Israel. Information Professor J Sadé, International Conference on Cholesteatoma and Mastc

Tel-Aviv Israel.

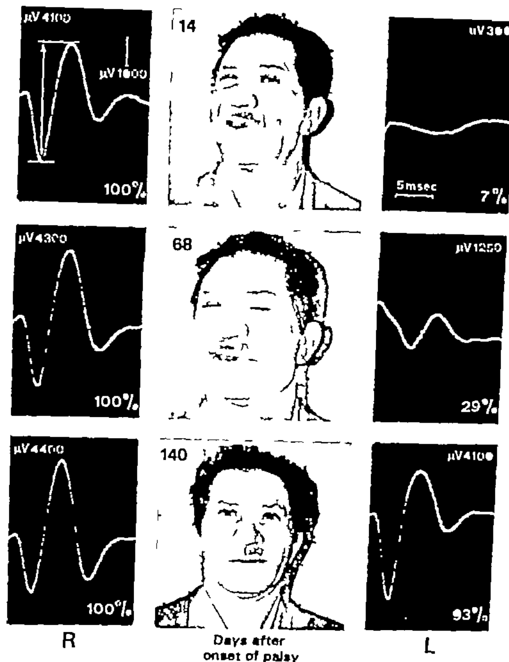


Fig 1 Electroneuronogram of a patient with Bell's palsy

tion block since only the former are meaning ful with regard to prognosis and hence in the choice of treatment

MATERIAL AND METHOD

The examination is performed with a Mk III Amplaid an apparatus commonly employed in the study of evoked acoustic potentials plus a stimulator with its accompanying system of stimulus and recording electrodes

The stimulus consists of a 0.2 msec 50 to 120 V rectangular pulse Stimulation starts with near zero voltage pulses at a frequency of 1 per sec and progresses to the point of maximum stimulus i e when no further in crease in response can be detected on the oscilloscope

The stimulation must be able to activate all the nerve fibres and an intensity of 5–10% more than the maximum is therefore em ployed This value depends on certain patient variables such as age and the development of the muscle masses It will obviously be retained in all subsequent sessions

The surface bipolar stimulation electrode is

of urgency Rapid efficient and dependable diagnostic techniques are essential therefore

Electroneuronography is able to satisfy these requirements in a particularly effective manner By comparison with other electrodiagnostic methods it has the prime advantage of offering early exact data that can be readily translated into quantitative values If the nerve deficiency is more than 90% for example surgery may be undertaken less than 72 hours after the onset of traumatic paralysis since the simplicity and rapid execution of the test enables what amounts to a continuous watch to be kept on any progressive degeneration of the nerve

Evaluation, of course will depend on the aetiology However once it is remembered that a facial defect does not become clinically evident until more than 40% of fibres cease to function a test capable of detecting changes of only 5% is clearly a valuable guide in assessing both the further progress of a paralysis and the effects of treatment.

False negatives or positives are of course inevitable in all forms of diagnostic enquiry Decisions with respect to treatment therefore should definitely be taken in the light of the evidence provided by several examinations which should be repeated in further sessions wherever possible

ZUSAMMENFASSUNG

Die Elektroneuronographie (ENoG) besteht in der Aufzeichnung eines durch einen supramaximalen elektrischen Reiz hervorgerufenen Summationspotentials der motorischen Einheiten. Die Degeneration (und offensichtlich die Unterbrechung) einer Nervenfaser bedeutet immer die Denervierung aller sich auf dieselben beziehenden Muskelfasern, so daß die Amplitudenabnahme des Summationspotentials der Anzahl der enervierten motorischen Einheit verhältnisgleich sein wird. Dem Test liegt der Vergleich der Gegenwirkungen, je Reiz gleicher Stärke zwischen gesunder und kranker Seite zugrunde, was ermöglicht, die Anzahl der Nervfasern des verletzten Teils anteilmäßig festzustellen, die gegenüber dem unbeschädigten Teil vermögen das Zusammenziehen der davon abhängigen Muskelfasern hervorzurufen. Der Verfasser schildert die bei 60 persönlich beobachteten Fällen erzielten Ergebnisse und bespricht die unleugbaren von diesem einfachen elektrodiagnostischen System gebotenen Vorteile bei der Beurteilung der Entwicklung einer Paralyse des Gesichtsnervs und bei der Festlegung genauer und zeitiger therapeutischer Angaben vor allem vom chirurgischen Gesichtspunkt aus.

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Podvinec, M 1977 Electroneuronography in prognosis of intratemporal facial palsy as compared to measurements of rheobase and chronaxy *ORL* 39 185

Prof G Rossi
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DISCUSSION

Stahl to Rossi Have you any experience of measuring the area of the entire reaction besides the amplitude

Jakobi to Rossi Die guten Erfahrungen können wir nur bestätigen, meinen aber mit M. Portmann, dass weitere Parameter zur Beurteilung eines Verlaufes de-Facialisparese notwendig sind Der Fingerdruck, mit dem Rossi die Elektroden halten, lässt können eine Fehlerquelle sein. Wir bevorzugen deshalb für unsere sehr viel kleineren Elektroden den Bestonukleber

Pfaltz to Rossi A single response in neuronography is not very reliable For this reason averaged responses ought to be used for more reliable evaluation of the global facial response

Ehrenberger to Rossi. Après nos expériences la neuronographie est une méthode éprouvée en cas des paralyses de Bell, mais pas en cas des paralyses traumatiques: même une perte totale de la réaction pendant la première semaine peut être suivie d'une restitution totale de la fonction nervale après plusieurs semaines.

Rossi (Reply)

Electroneurography is a very useful test in the diagnosis of facial palsy Yet it is by no means the only electrical

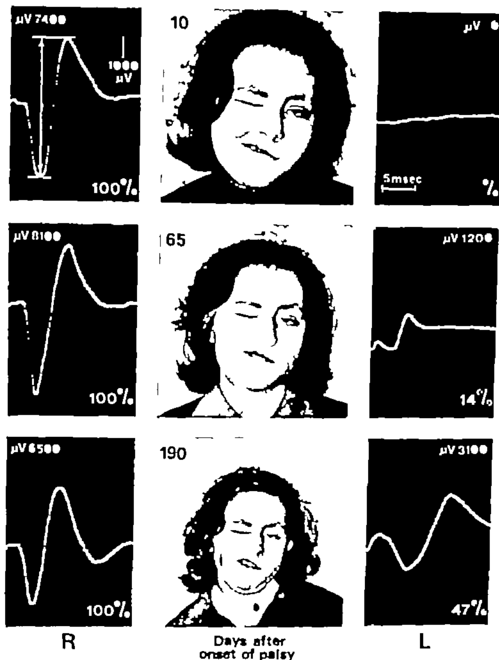


Fig 3 Electroneuronogram of a patient with intratemporal traumatic lesion of the facial nerve and with facial nerve graft.

Diagnosis is based on repetition of the test at intervals determined by the situation and on evaluation of the increase or decrease in response observed Variations of as little as 5% between one examination and another can be detected

CASE MATERIAL

Our series consist of 60 cases 28 Bell's palsy 10 Ramsay Hunt palsy 6 postparotidectomy paralysis 12 post-traumatic paralysis 2 post traumatic paralysis with nerve graft in the intramastoid tract and 2 essential spasm (Figs 1 2 3)

DISCUSSION AND CONCLUSIONS

Electroneuronography is a useful means for monitoring the progress of paralysis Its most valuable contribution however is to the early diagnosis of diseases of the facial nerve

Treatment of facial paralysis particularly surgery must in fact be regarded as a matter

of urgency. Rapid, efficient and dependable diagnostic techniques are essential therefore.

Electroneuronography is able to satisfy these requirements in a particularly effective manner. By comparison with other electrodiagnostic methods it has the prime advantage of offering early exact data that can be readily translated into quantitative values. If the nerve deficiency is more than 90% for example surgery may be undertaken less than 72 hours after the onset of traumatic paralysis since the simplicity and rapid execution of the test enables what amounts to a continuous watch to be kept on any progressive degeneration of the nerve.

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False negatives or positives are of course inevitable in all forms of diagnostic enquiry. Decisions with respect to treatment therefore should definitely be taken in the light of the evidence provided by several examinations, which should be repeated in further sessions wherever possible.

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Electroneurography is a very useful test in the diagnosis of facial palsy. Yet it is by no means the only electrical

diagnostic test that need be employed Its results must always be supplemented by those obtained by chronaximetry evaluation of the rheobase and electromyography

Desynchronization of the response is often noted when the patient is examined more than 30 days after the onset of paralysis The difference sometimes noted between the amplitude of the response and the extent of functional recovery is probably attributable to such desynchronization

Like all previous authors in this field we evaluate the amplitude of the potential only Latency and the area of the wave are of scant diagnostic or prognostic interest

We always soak our electrodes in saline because the are flat and not of the suction-cup type

When palsy is due to trauma functional recovery may be noted a few days after the injury This is the case when concussion is involved since the phenomena to which it gives rise can be resolved in a few days if suitably treated, and does not represent an interruption of the nerve fibres. *If the fracture site cannot be located radiographically* therefore it is better to wait 15 days before embarking on surgery This in turn will need to be undertaken after the 10th day when daily repetition of all the various electrodiagnostic tests has led to negative results for at least 4–5 days

Acta Otolaryngol 89: 277–282 1980

THE R THRESHOLD OF THE QUICK COMPONENT

A New Measure in Electronystagmography

M Pansini and I F Padovan
Zagreb Yugoslavia

Vestibular imbalance affects the eyes through the fasciculus longitudinalis medialis and oculomotor nuclei As regards vestibular excitation, eyes could theoretically be turned around and around until balance is achieved However since physical and anatomical limitations do not allow such turning around the eyes could be held in the utmost lateral position in the position of conjugated deviation Nevertheless the eyes are not held in this position of conjugated deviation since the compensatory central component brings the eyes back to the position of azimuth zero Logically this should depend on physical limitations and on the tension of the eye muscles which transmit information to the central structures via the proprioceptive afferent paths The speed of the slow component and other elements also plays a certain role here It seems that we are faced with a great task of determining all these elements in order to define the point where the returning quick eye movement begins

There are many elements which send commands to revert eyes. Sometimes the eye reverts after a high amplitude and sometimes after a low amplitude It is not the same in all individuals (Collard & Eber 1977) Many central mechanisms which are involved are not yet fully understood The number of the unknown elements is too large to allow any possibility of their definition and measurement. (Fig. 1)

However the first step was carried out by determining the point where the slow component of nystagmus stops and the quick component begins as the point where the mechanism for reverting the eye starts to work. This point could be called the threshold of the quick component This means that the point is identified but not explained Its identification makes it a possible subject for investigation

Identification and Measurement of R

The assumption that this point can be defined only by knowing all the elements which determine it, is quite discouraging. It *is* possible however to make a comparison between the many elements of nystagmus which are measurable even though their mechanisms are still not well known We may say that it is not absolutely necessary to know *all* the mechanisms in order to determine and measure this threshold On the contrary by making the threshold of the quick component measurable it serves for purposes of investigation and learning about the elements which determine it

The next step in our exploration was made when we assumed that if there is a certain threshold of sensitivity for the appearance of the quick component, then it must be *constant* in the examined individual no matter whether nystagmus is weak or strong whether it is at the beginning in the middle or at the end of the provoked nystagmus. This is called *the* law of preservation The Nobel Prize winner physicist Feynman says to a physicist, the law of preservation means the existence of a number which is definable at a certain moment, and if we define it again after some time the number will not change—it will be of the same value no matter what changes occurred in the meantime

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The threshold of the quick component was

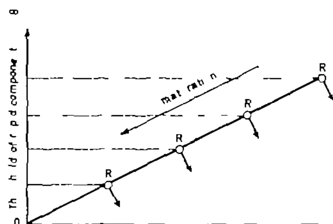


Fig 1

Table I

f	s	R
3 1	25	2.6
1 7	6	2 1
.2	11	2.3
1 6	4.5	1 7
1.3	3.5	2.1
0 9		2.5
1.2	2.8	1 9
3	19	.1
2.5	14	2.2
1	3	3
3.	29	.8

to be obtained as a numerical datum from al ready existing elements of nystagmus In a nystagmic jerk we distinguish duration t amplitude a slow component l and quick com ponent r If as in geometry we mark sides with small letters and vertexes with capital ones then the threshold of the quick compo- nent is the point R (Fig 2)

After defining the threshold of the quick component and being convinced that R does not change in one measuring in a rather short time it was possible to establish that $R=s/f^2$ where s represents the speed of the slow com ponent and f the frequency of nystagmus In the actual nystagmogram it was shown that changes in frequency f and the speed of the slow component s do not cause significant changes in the threshold of the quick compo- nent R which confirms the value of the above cited mathematical formula (Table I)

It was possible to confirm the value of the formula on a model and also to show that the value for the threshold of the quick component has a span from zero to an indefinite number It also shows a precision by which even small changes can be detected (Fig 3)

Formula $R=s/f^2$ worked well in practice although it was not clear what its basis was It was hoped that mathematics which is the foundation of all things and mechanisms could sometimes solve such problems by it self So it happened that by substituting a for s (from the old formula $s=a \times f$) formula $R=s/f^2$ transformed into $R=a/f$ (Table II)

Now it was all very clear and simple The threshold of the quick component will be greater if the amplitude is greater (it is directly proportional to the amplitude) and it will be smaller when the frequency is greater (it is conversely proportional to the frequency f) $R=a/f$

For practical purposes it is better to use for mula $R=s/f^2$ than the $R=a/f$ and for easier cal culation the following transformation can be used $R=(s\ f)\ f$ The speed of the slow com- ponent is divided by the frequency and the result is again divided by the frequency which gives the value of the quick component

In the 10 sec duration of nystagmus the number of nystagmic jerks is counted and divided by 10 which gives the value of fre quency to one decimal place

In the same section of the electronystagmo- gram we select three speeds of the slow com- ponent and calculate the average The average

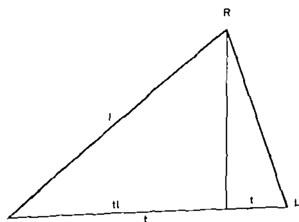


Fig 2

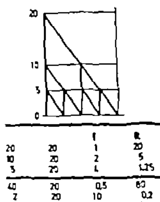


Fig. 3

Table II

$R = \frac{s}{f}$
$= f$
$\frac{L}{f}$
$R = \frac{\frac{L}{f}}{\frac{f}{f}} = \frac{1}{f^2}$
$R = \frac{1}{f^2}$

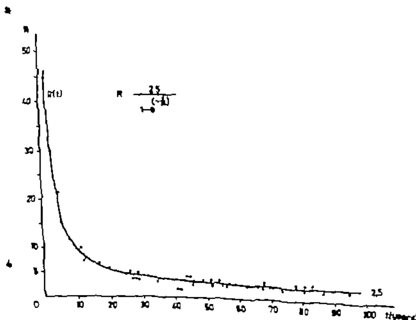
of the slow component is then divided by the frequency and this result is again divided by the frequency according to the formula $R = (s/f)/f$

Vestibular Maturity and R

We may ask if this datum has any meaning, considering the fact that it only measures the threshold of the quick component and does not determine the elements which caused it. To defend the value of threshold of the quick component we list now some possibilities which are available for use

We took a large number of electronystagmograms of healthy individuals aged between 4 and 76 and measured R in each of the four caloric tests. Data were put on a graph where axis x carries the values for age and axis y the values of R (Fig. 4)

The line connecting the points which represent the average values shows a curve descending as age increases. This curve is regular in shape so we asked our electronics en-



gineer Mr Kozina to provide a formula for it which is as follows

$$R = \frac{2\ 5}{1 - e^{(-\pi)}}$$

where

R = threshold of the quick component

e = natural logarithm

t = age

This means that with increasing age *R* de creases and tends towards the value 2 5 When this formula was fed into the computer it produced values for age between 0 and 100 (Table III)

We obtained this curve and formula as well as the table on the basis of the specific num ber of examinations of healthy individuals which could differ in a new investigation However we believe that mathematically the purest curve and formula would be the one which anticipates that *R* is infinite at the moment of conception (Tibbling 1969) while *R* is zero at the infinite age Then we could say for the individuals with pathologically small *R* how many hundreds of years their results correspond to or we could calculate the *R* of Adam who lived 930 years or Methuselah the oldest man who lived 969 years[1]

When measuring *R* in certain individuals, we can say whether it corresponds his/her age taking of course into account certain physiological limits of +/−20%

The threshold of the quick component certainly depends on myelinization and generally on the maturity of the central nervous system, thus a bigger R in children suggests vestibular immaturity We say vestibular immaturity because we think it refers primarily to the area of reticular formation and vestibular nuclei ([illegible] & Jeannerod 19[illegible]) It is also possible that there are cerebral influences from different regions, especially from the frontal and [illegible] area, the cerebellum, labyrinthal proprioceptive and [illegible] areas as well as from the [illegible] nuclei, fasciculus longitudinalis medialis and nuclei Cajal and Darkschewitsch [illegible] ([illegible])

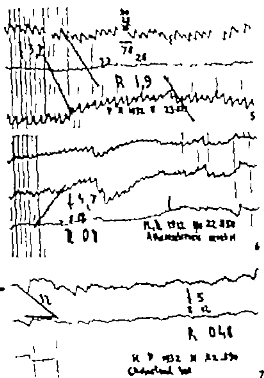
Table III

t (years)	*R*	*t* (years)	*R*
0			
1	88 76	51	3.26
2	45 01	52	3.[illegible]3
3	30 43	53	3.20
4	[illegible]3 15	54	3 18
5	18 78	55	3 16
6	15 87	56	3 13
7	13 79	57	3 11
8	12.[illegible]4	58	3 09
9	11 03	59	3.07
10	10 06	60	3.05
11	9.27	61	3.03
12	8 61	6[illegible]	3.01
13	8.06	63	3 00
14	7.57	64	[illegible].98
15	7 17	65	2.96
16	6.81	66	2.95
17	6.50	67	2.93
18	6.22	68	2.9[illegible]
19	5 97	69	[illegible].90
20	5 74	70	[illegible].89
21	5.54	71	[illegible].88
[illegible]	5.36	72	[illegible].87
23	5 19	73	[illegible].85
24	5 04	74	2.84
25	4.90	75	.83
26	4 77	76	[illegible].82
27	4 65	77	[illegible].81
28	4.54	78	[illegible].80
29	4 44	79	[illegible].79
30	4.34	80	78
31	4.25	81	[illegible]77
3[illegible]	4 17	82	2.77
33	4 10	83	[illegible] 6
34	4 02	84	[illegible].75
35	3 95	85	[illegible] 4
36	3.89	86	[illegible].73
37	3.83	87	[illegible].73
38	3 77	88	72
39	3 [illegible]	89	[illegible].71
40	3 67	90	[illegible] 1
41	3 6[illegible]	91	[illegible].70
42	3.58	92	[illegible].69
43	3.53	93	[illegible].69
44	3 49	94	[illegible].68
45	3 46	95	[illegible].68
46	3 42	96	[illegible].67
47	3.3[illegible]	97	[illegible].67
48	3 35	98	[illegible].66
49	3 32	99	[illegible].66
50	3.29	100	[illegible].65

Clinical case

From a child with leukoencephalitis, we obtained a *R* which was larger than the one that normally corresponds to his age (13 yrs) As

[1] Methodology which could evaluate neurological [illegible] has not existed till now



Figs 5–7 5 A case of atherosclerosis, with $R = 1.9$ 6 Another case of central atherosclerosis ($R = 0.8$) 7 A patient whose first measured R value was 0.5 It was subsequently found that his cholesterol value was 400

the illness progressed R increased to reach 64 in few months.

Angiopathies and R

In our investigations we noticed that the value of the threshold of the quick component decreases in central angiopathies It is less than 2 and in greater disturbances it is less than 1 (Figs 5–7)

Possibilities open to Investigation

Our investigations until now were limited only to the following two points (1) description and use of the new element in the electronystagmogram viz. the threshold of the quick component, and (2) explanation of the possibility of measuring this threshold and expressing its value numerically

Everything else presented in this communication is only the beginning of the work which in our opinion promises significant diagnostic possibilities

As an example for investigation we would like to mention a formula for fixational suppression

$$FS = \frac{NF\,(\text{non-fixation}) - F\,(\text{fixation})}{NF\,(\text{non-fixation})}$$

where usually the speed of the slow component is worked with but it is also possible to use the threshold of the quick component R We did it while examining a few healthy individuals and obtained the same values for the fixational suppression in s and R

Fixational suppression expressed in s was 85 1 and in R it was 85 7 It is interesting because in some pathological cases regarding fixational suppression there was no connection between values of the speed of slow component and the threshold of the quick component. It means that it is possible to discover disturbances in some mechanisms of fixational suppression and surely there *is more than one*

It is easy to construct a formula for calculating R in each individual nystagmic jerk We adjusted it to the speed of the ENG sheet of 15 mm/sec while for the speed of 10 mm/sec we should substitute the number 10 for 15

$$R = \frac{S}{\left(\frac{15}{N}\right)} \quad \text{or } R = \frac{S}{\left(\frac{10}{N}\right)}$$

N = length of the nystagmic jerk, in mm

$\frac{15}{N}$ or $\frac{10}{N}$ = frequency of 1 jerk

This is a new datum which can help in the exploration of nystagmic irregularities where the change in threshold of the quick component is mostly involved

Various influences and effects on the threshold of the quick component can be explored effect of sedatives influence of the various levels of intelligence and motorics relationship with neurological diseases especially

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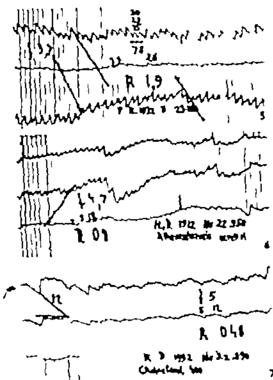
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0			
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2	45.01	52	3.23
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4	23.15	54	3.18
5	18.78	55	3.16
6	15.87	56	3.13
7	13.79	57	3.11
8	[illegible]	58	3.09
9	11.03	59	3.07
10	10.06	60	3.05
11	9.27	61	3.03
12	8.61	62	3.01
13	8.06	63	3.00
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26	4.77	76	2.82
27	4.65	77	2.81
28	4.54	78	2.80
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31	[illegible]	81	2.77
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36	3.89	86	2.73
37	3.83	87	2.73
38	3.77	88	2.72
39	3.72	89	2.71
40	3.67	90	2.71
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42	3.58	92	2.69
43	3.53	93	2.69
44	3.49	94	2.68
45	3.46	95	2.68
46	3.4	96	2.67
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Various influences and effects on the threshold of the quick component can be explored effect of sedatives influence of the various levels of intelligence and motorics relationship with neurological diseases especially

those causing demyelinization and also the relationship between deafness and the rehabilitation of hearing

CONCLUSION

Nystagmus is not a simple vestibulo-ocular reflex but one that passes through areas of the central nervous system which imprints its messages into provoked nystagmus This is evident in an ENG recording Reading those central messages and learning about this central semiology is the most significant area of electronystagmography today We hope that *R* the threshold of the quick component will as a new measure also provide new possibilities for further explorations in this field

ACKNOWLEDGEMENT

My co-worker Professor M Pansini began this investigation 4 years ago in our Department where our Laboratory for Vestibulogy has gained extensive experience in electronystagmography Our electronics engineer Mr Kozina, assisted us greatly with our mathematical formulas and data processing Our work is only preliminary but we can now confirm that these present results will not only be a subject for investigation but also be useful and applicable for routine clinical work

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DISCUSSION

Tonndorf to Padovan Nystagmus, as is generally known is a very noisy signal Therefore a method of noise reduction is needed the simplest being averaging. Now with a time varying signal the time over which one averages—the time window—is important. It must not be too short but not too long either What was your method of evaluation?

Jongkees to Padovan. What is the meaning of the term threshold of the quick component" during a caloric test. It is certainly not so that the quick phase always re erts the eye to a mid-position It can even take it further away from it.

Pfaltz to Padovan Your hypothesis is based on the results of repeated caloric tests What parameter did you use to determine the threshold of a caloric response

Manni to Padovan I do not agree that the quick phase of eye nystagmus originates from conjunctiva receptors or from muscular proprioceptors. The source of the quick phase is located in the brain stem mainly in the reticular formation However the eye nystagmus may be influenced by trigeminal afferents.

Padovan (Reply)

To Tonndorf In 10 sec of the most regular nystagmus we determined the frequency the three rates of slow component and then the average value of the slow component No significant differences were observed in any of the 10 sec The values of R were as a rule similar in four caloric tests When we measured R we measured it in all four or six caloric tests.

To Jongkees We are interested in the phase the quick component begins Whether the quick component of the eye approaches reaches or passes the mid-position has an effect on the amplitude and a partial effect on the speed of the slow component which we observed in the recording. (However the correct speed of slow component is only when the eye reaches mid-position)

All of these and other changes may have a significant effect on R so that R is as unreliable as the amplitude, frequency and rate of slow component, which we likewise measure and consider important for the diagnosis R is neither a more or less precise measurement than the others

We call R the threshold of quick component, because a certain strength in the mechanism of the central component is necessary for the eye to return This is not the same in each of the examinees. We call this reaction the threshold in the same way we do in the measurement of the stapedial reflex

To Pfaltz In routine caloric tests we use the a erage value of the rate of the slow component obtained in 10 sec of the strongest response We do not determine the threshold of the caloric response in the caloric test

To M nni We agree with the majority of authors that the quick phase originates in the reticular formation, that it is likewise influenced by trigeminal afferents and that many other influences certainly exist Howe er we do not consider these obstacles for determining and measuring R since we could reject all other measurements in electronystagmography for the same reasons. We ha e suggested R and its importance can be determined by all those who use t in their work.

Acta Otolaryngol 89: 283–294, 1980

IMMUNODEFENCE OF THE INNER EAR?

Lymphocyte–Macrophage Interaction in the Endolymphatic Sac

Helge Rask Andersen and Jan Stahle

From the Department of Otolaryngology, University Hospital, Uppsala

Abstract. Owing to their proximity to areas exposed to infection, the sensory organs of the inner ear are probably dependent on an efficient antimicrobial defence. The longitudinal flow of endolymph to the endolymphatic sac may be of major importance in this context. Substances entering the ear can be automatically carried to the distal part of the endolymphatic duct where lymphoid cells are present and endolymphatic phagocytosis occurs. In the intermediate part of the epithelium of the sac morphological signs marking the incoming substances are present. A vigorous interaction between lymphocytes and macrophages, similar to that observed in antigen-activated lymphoid tissue, may be seen. The sac is here surrounded by a rich network of lymphatic capillaries and blood vessels.

The endolymphatic duct and sac are believed to absorb endolymph. Guild stated in 1927 that endolymph flows from the cochlear duct to the endolymphatic sac where it is absorbed and mediated to the numerous perisaccular blood vessels.

Phagocytosis by both the epithelial cells and free cells of the endolymphatic sac was demonstrated experimentally by Andersen (1948), Engström & Hjort (1950), Arnvig (1951), Lundquist (1965) and Rudert (1967).

The purpose of a phagocytic cell system inside the endolymphatic space is not known. Does the sac receive from the labyrinth effete cells or metabolic waste products too large and toxic for disposal via the cochlear duct? Or do the freely floating cells cleanse the sac of proteins normally concentrated therein and transport them via the epithelium into the surrounding blood vessels, as proposed by Ishii et al. (1966)?

Guild defined twenty-two species of cell element in the lumen of the endolymphatic sac. These included lymphocytes whose presence prompted Lim & Silver's (1974) proposal that the endolymphatic sac acts as an immunodefensive organ for the inner ear. Since toxins or foreign substances could reach the labyrinth through the windows of the middle ear cavity it could be crucial to inner-ear homeostasis that these substances are removed and eliminated by phagocytic cells in the presence of immuno cells.

In the present investigation, lymphocytes and macrophages in the endolymphatic sac of guinea pigs were studied ultrastructurally. This was done in order to reveal a possible physical interaction which would indicate functional cooperation and could thus confirm the theory that the endolymphatic sac acts as an immunodefensive organ for the internal ear.

MATERIAL AND METHODS

Fifty pigmented guinea pigs ranging in weight from 200 to 800 g were used in this investigation. The animals displayed a normal Preyer reflex. They were anaesthetized with sodium pentobarbital (Nembutal) and perfused with a solution of 2.5% (v/v) purified glutaraldehyde and 1% (v/v) formaldehyde in a solution of 0.1 M Sørensen's phosphate buffer, pH 7.2–7.4. After decapitation the middle and internal ears were examined macroscopically for signs of infection. The operculum covering the endolymphatic sac was eliminated with a fine

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Supported by the Swedish Medical Research Council Project No. 03908.



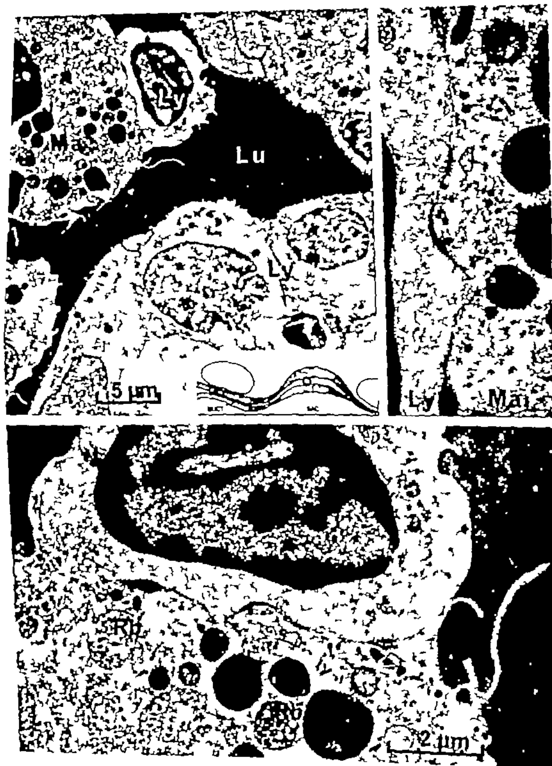
Fig 1 Light micrograph demonstrating the intra-osseous ES in the guinea pig A few free luminal cells are seen in the darkly stained endolymph of the proximal sac where the endolymph appears vacuolated The number of free luminal cells is increased towards the rugose portion of the sac where a large number of cells approach the epithelium and the non-vascularized epithelial process (*NVP*) *Inset*: higher magnification of the NVP clearly reveals its unique structure as compared with the rest of the epithelium. Note its close association with the free luminal cells

needle and the endolymphatic sac (ES- specimens displayed and dissected from the temporal bones The specimens were immersed in the same fixative for 24 h post fixed in 1% OsO_4 dehydrated in alcohol and propylene oxide embedded in Epon 812 and trimmed and sectioned on an LKB ultratome Ten temporal bones were decalcified in 0 1 M Na EDTA and 2 5% purified glutaraldehyde in Na-cacodylate buffer for 14 days The bones were serially sectioned both parallel and perpendicular to the long axis of the endolymphatic duct and sac Semi-thin sections (1–2 μm) were stained with toluidine blue or *p* phenylenediamine while ultrathin sections were contrast stained with uranyl acetate and lead citrate The specimens were examined and photographed in a JEOL 100 B electron microscope

OBSERVATIONS

Earlier research on the vascular anatomy and supply of the endolymphatic duct and sac (Rask Andersen 1979) revealed an extensive vascular network of arteries capillaries venules and veins surrounding the endolymphatic sac while the endolymphatic duct was relatively poorly supplied with blood vessels The endolymphatic duct is surrounded by a fairly dense network of lymph vessels which show a characteristic arrangement suggesting their participation in endolymph fluid transport (Rask Andersen & Bredberg 1979) These lymph capillaries lack a typical basal lamina and almost invariably display interendothelial gaps often facing the lumen of the endolymphatic duct The lymph vessels surrounding

Fig 2 *Top left* Two lymphoid cells in the intermediate portion of the ES One lymphocyte (*arrow*) lies in the epithelium while nother cell (*Ly*) is attached to the cell surface of a large intraluminal macrophage (*Ma*) ×6300 *Bottom* The lymphocyte adheres to the cell coat of the macrophage which forms cytoplasmic protrusions embracing the lymphocyte ×14500 The cytoplasm of the macrophage is filled with granular residual bodies (*Rb*) and darkly stained organelles. *Top right* Higher magnification showing the close contact between the lymphocyte and macrophage Note areas of subplasmalemmal densities ×14000


Ly
M
Lu
Ly
5 µm
Ly
Ma
2 µm

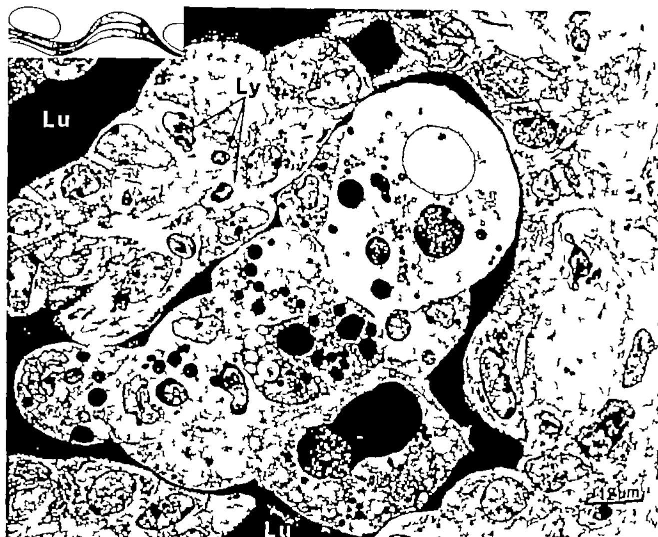


Fig 3 A cell cluster in the luminal space (*Lu*) approaches the villous epithelium of the rugose endolymphatic sac and is trapped in a deep crypt The epithelium is infiltrated by lymphoid cells (*Ly*) The cell cluster probably coalesces with the epithelium to form a non-vascularized epithelial process ×4 900

the endolymphatic sac are devoid of interendothelial gaps Our studies suggest that the endolymphatic duct is concerned with fluid resorption while the sac is engaged in phagocytosis

The proximal endolymphatic sac contains a viscous osmiophilic endolymph composed of clear vacuoles and particles This precipitated endolymph is phagocytosed or engulfed by incoming macrophages or luminal histiocytes (LH) whose cytoplasm becomes filled with endolymph while the cells assume a signet ring appearance Lymphocytes can be observed among these freely floating cells which later coalesce to form cell clusters Many degenerate and appear to be transported distally in the endolymphatic sac (Fig 1)

Lymphocyte–macrophage interaction

Macrophages and lymphocytes are intimately associated in the luminal space The mac

Fig 4 Electron micrographs of the rugose portion of the ES (A) An epithelial cell process (*NVP*) project into the electron-dense endolymph which houses a few macrophages The NVP is bordered by an irregular epithelium the cell cytoplasm of which often appears vacuolated Among the epithelial cells are scattered polymorphonuclear leukocytes (*PMN*) a few monocytes (*M*) and numerous macrophages containing a number of granular residual bodies The cells are closely apposed to each other Adjacent columnar cells show fragmentation of the apical cytoplasm, and cytoplasmic blebs have discharged into the endolymph. ×1 500 (B) The dark endolymph ( ) contains huge binucleate amoeboid cells showing phagocytic properties A lymphocyte (*Ly*) (*left*) migrates into a narrow epithelial crypt while another cell (*right*) lies close to the cell surface of a macrophage (*Ma*) ×1 100



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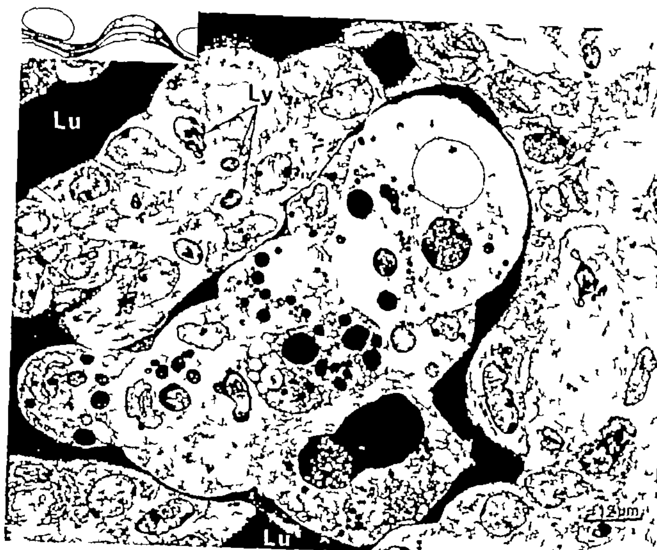


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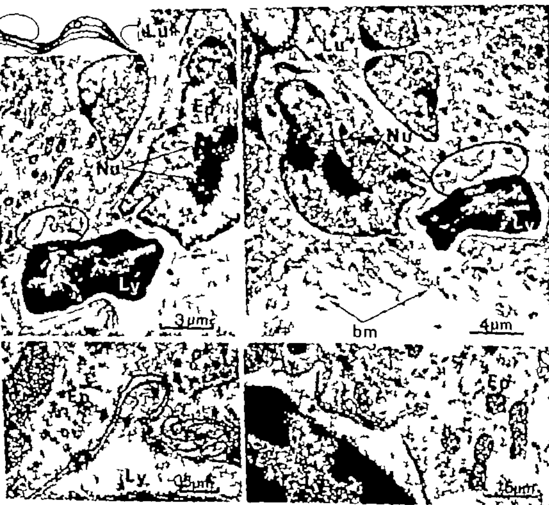


Fig 6 Lymphocytes (Ly) in the rugose epithelium of the ES *Top* The cells adhere to the epithelial cells (*Ep*) which display two nucleoli (Nu). *Lu* Endolymphatic lumen. ×2400 (*left*), ×1750 (*right*). *Bottom* Pseudopodial processes project into the cytoplasm of the epithelial cells. There are small areas of subplasmalemmal density ×9800 (*left*), ×5400 (*right*)

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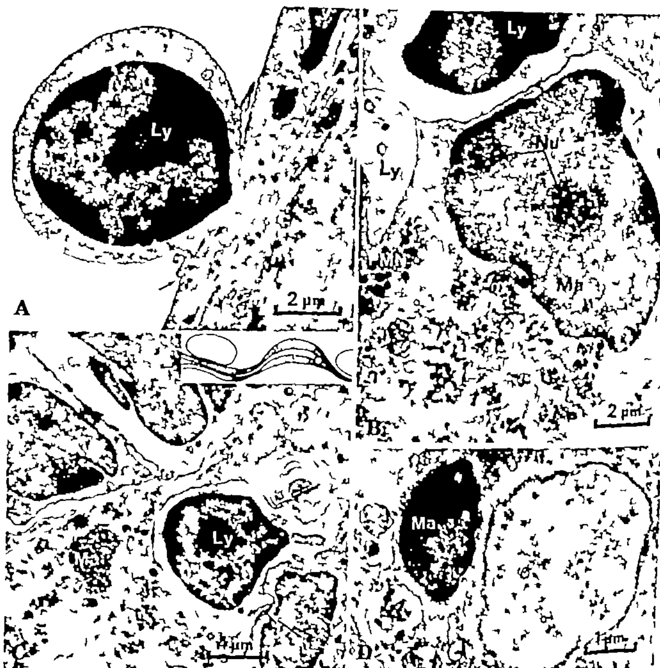


Fig 5 (A) A lymphocyte (*Ly*) migrates through the thin endothelial cell of a post-capillary venule into the loose stroma around the rugose wall of the ES Endothelial cell junction (*arrow*) ×6600 (B) Macrophage–lymphocyte association in a NVP of the ES The macrophage contains numerous residual bodies The large nucleus has two nucleoli (V) There is a broad area of close contact between the cells ×9000 (C) The lymphocyte has penetrated deep into the cytoplasm of a macrophage (*Ma*) which is rich in intracytoplasmic residual bodies A small uropod-like cytoplasmic protrusion of the lymphocyte is oriented peripherally ×3100 (D) One cell in a NVP has penetrated deep into the cytoplasm of a macrophage (*Ma*) and appears to be located intracellularly due to oblique sectioning *Rb*=Residual body ×11 1?0

rophages contain residual bodies with large amounts of digested material There are areas of close contact between the cells with reduced intercellular distance The plasma membrane shows numerous coated pits and vesicles while dendritic cytoplasmic projections encompass the lymphoid cells holding them close to the macrophages (Fig 2)

Non vascularized epithelial processes (NVP)

NVP could be observed in all animals investigated These epithelial processes in the distal



Fig 6 Lymphocytes (*L*) in the rugose epithelium of the ES *Top* The cells adhere to the epithelial cells (*Ep*) which display two nucleoli (*N*). *Lu* Endolymphatic lumen. ×2 400 (*left*), ×1 750 (*right*) *Bottom* Pseudopodial processes project into the cytoplasm of the epithelial cells. There are small areas of subplasmalemmal density 9 800 (*left*) 5 400 (*right*)

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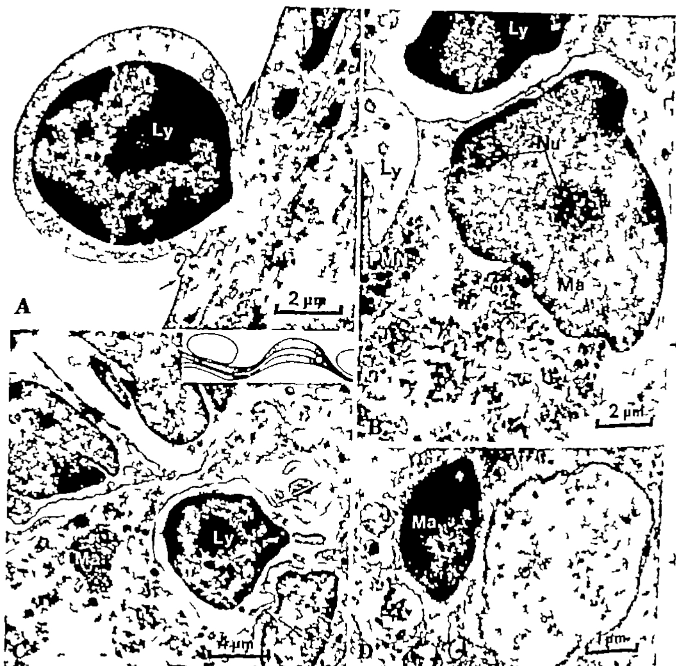


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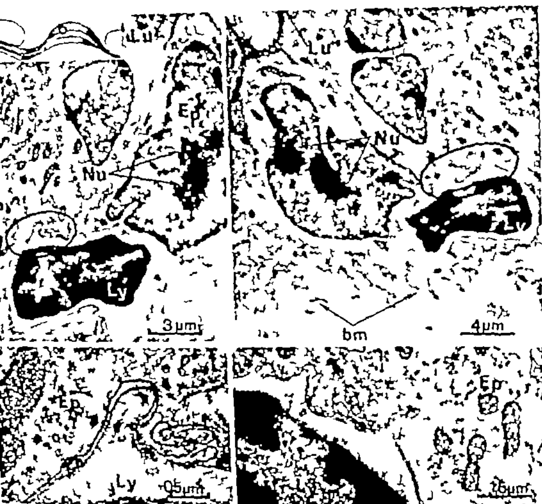


Fig. 6. Lymphocytes (*Ly*) in the rugose epithelium of the ES. *Top:* The cells adhere to the epithelial cells (*Ep*) which display two nucleoli (*N*). *Lu:* Endolymphatic lumen. ×2400 (*left*), ×1750 (*right*). *Bottom:* Pseudopodal processes project into the cytoplasm of the epithelial cells. There are small areas of subplasmalemmal density. ×9800 (*left*), ×5400 (*right*).

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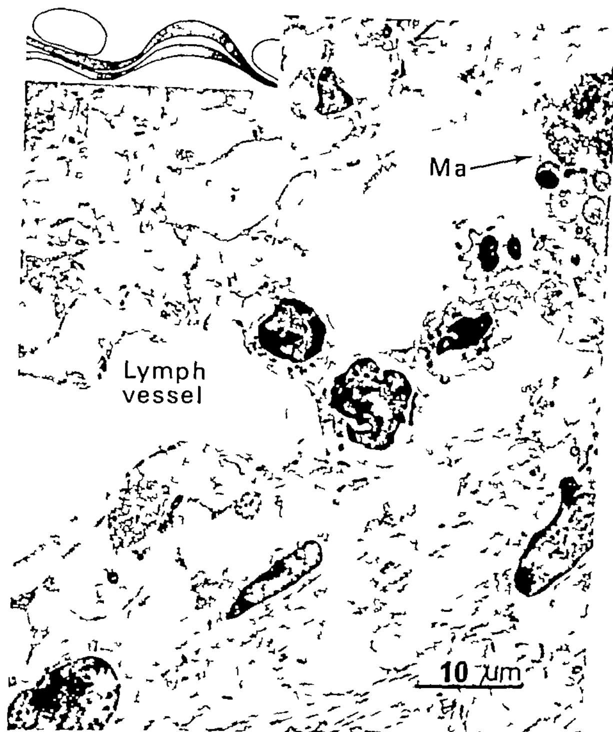


Fig 7 A lymph vessel situated between the intermediate and distal portions of the ES It contains large lymphocytes and macrophages filled with *condensed material* (*Ma*) The slender lymph vessel lacks typical basement membrane ×2000

Lymphocytes and sac epithelium

Lymphocytes are found in the stroma surrounding the intra-osseous sac Many take on a more primitive or blastoid appearance with a large translucent cytoplasm their nuclei frequently containing a prominent nucleolus Some cells appear to migrate into the epithelium and assume a position close to the dark columnar epithelial cells (Fig 6) The nuclei of the dark cells adhering to lymphocytes display conspicuous nucleoli and in places the plasma membranes are thickened into what appear to be contact sites between the cells No cytoplasmic bridges are found



Fig. 8. Plasma cells and lymphocytes in the distal region of the sac. This region of the perisaccular tissue is so rich in plasma cells in some animals that it may be called the 'plasma-cellular' region of the ES. *Top left:* Two mature plasma cells (*PC*) contain large amounts of rough endoplasmic reticulum and characteristic prominent perinuclear Golgi structures. Lymphoid cells appear as 'blastoid' cells and are scattered nearby. 1200. *Top right* and *bottom left:* The plasma cells (*PC*) have close relationship to the reticular connective tissue cells (*R*). Numerous coated pits and vesicles suggest macromolecular transport in the reticular cell cytoplasm. 5000 9400. *Bottom right:* Plasma cells (*PC*) surround large lymphocytes. Prominent mitochondria and multivesicular body are seen. 7500

Perisaccular tissue

The intra-osseous portion of the endolymphatic sac is surrounded by an extensive network of post-capillary venules and veins. It is not unusual to observe small lymphocytes migrating through the thin endothelial walls into the loose tissue (Fig. 5A). Mast cells are widely distributed, many of them with pseudopods

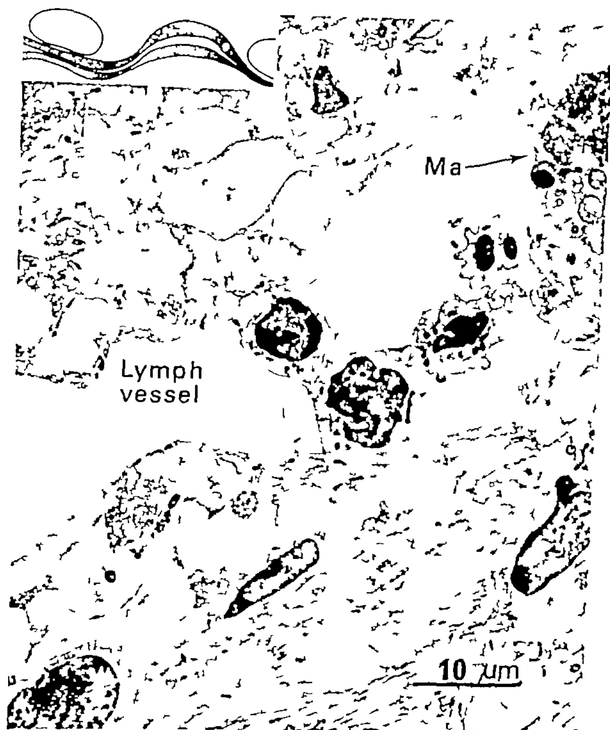


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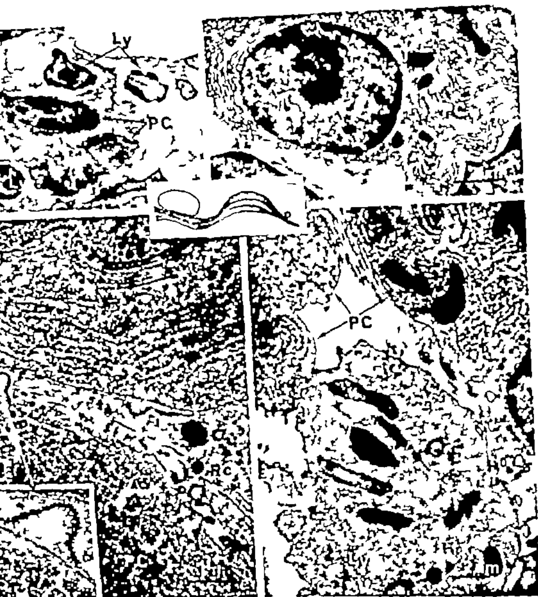


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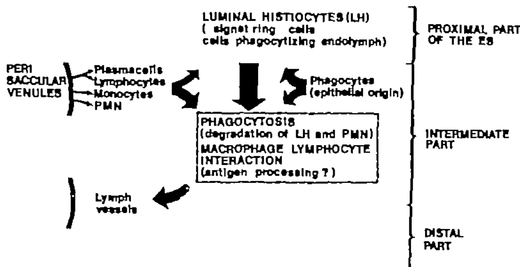


Fig 9 Hypothetical representation of the turnover of the free cells of the ES based on morphological observations in guinea pigs Intraluminal cells start to appear in the distal endolymphatic duct and proximal sac The cells ingest endolymph and assume a signet-ring appearance More distally many cells form cell clusters and present a more degenerated appearance Lymphocytes migrate through the thin walls of the perisaccular venules and appear around the luminal histiocytes (*LH*) Plasma cells monocytes and polymorphonuclear leukocytes (*PMN*) are observed in the subepithelial connective tissue Phagocytes originating from the intermediate sac epithelium are dispelled into the lumen to join the luminal histiocytes In the intermediate sac the epithelium is specialized into NVP where PMN and luminal histiocytes are being phagocytosed A close topographical relationship between macrophages and lymphocytes suggests the presence of an antigen-processing mechanism while the region between the intermediate and distal sac incorporates a well-developed lymph capillary network, often containing both macrophages and lymphocytes which probably indicate a continuous recirculation of lymphoid cells

Dendritic melanocytes rich in spherical melanosomes and stellate reticular cells are scattered among reticular fibres monocytes polymorphonuclear leukocytes and plasma cells

The endolymphatic sac is also surrounded by a prominent plexus of lymph vessels These slender vessels lack a typical basement membrane and are often filled with lymphoid cells and macrophages containing condensed intracytoplasmic material (Fig 7) The number of lymph vessels increases towards the distal region of the sac where a large plexus is found They are often of the same size as the neighbouring venules and veins The tissue around the NVP displays a conspicuously large number of mature plasma cells (Fig 8) They contain masses of endoplasmic reticulum with prominent Golgi complexes There are plasmoblasts and lymphoblastoid cells Indeed by reason of the large amount of plasma cells the region near the external aperture of the vestibular aqueduct could be called the plasmacellular area of the ES

DISCUSSION

Macrophages are found in many organs of the mammalian body and many probably originate from the bone marrow through the migration of monocytes from the circulation into the tissue where they are transformed into macrophages and even epithelioid cells and giant cells Nowadays considerable attention is focused on the macrophages during the immune responses Macrophages are able to trap and process antigen and appear to play an essential part in the presentation of antigen to immunospecific lymphocytes before the antigen response can become manifest (Cline & Swett 1968 Hersh & Harris 1968) Such functional co-operation may include intimate contact between the participating cells Morphological studies have demonstrated physical interaction between lymphoid cells in various lymphatic tissues (Sharp & Burwell 1960) and direct cytoplasmic bridges between lymphocytes and macrophages have been observed after challenge with antigen (Schoenberg et al 1964) In the ES in guinea pigs macrophage

lymphocyte associations are observed in both the luminal space and the NVP of the intermediate portion of the endolymphatic sac epithelium. Such associations are apparently not merely a rare coincidence of migrating lymphoid cells but represent actual physical interaction due to intercellular proximity presence of nucleoli and differentiation of the plasma membranes showing unspecific contact sites. This may represent functional cooperation between the cells. It is not known whether the cells are involved in any specific antigen-processing mechanism or immune response and this question needs to be investigated further. The close relationship between lymphoid cells and dark epithelial cells is interesting. According to Lundquist (1965) the dark cells of the intermediate sac are potential phagocytes which can probably be dispelled into the lumen to become freely floating cells. It is tempting to speculate that lymphocytes may interact also with epithelial cells before they differentiate into phagocytic cells.

An intricate complex interaction between immuno epithelial and phagocytic cells may in future be proved to promote phagocytosis in the ES.

On the basis of our morphological studies we devised a hypothetical model of the turnover of some of the free cells of the endolymphatic sac (Fig. 9). We postulate in principle two different kinds of phagocytic cell systems in the sac. One is composed of free cells (LH) appearing in the proximal sac which often assume a signet-ring appearance. The cells ingest large quantities of viscous endolymph and seem to be endolymph drinkers. The cells move distally to form cell clusters. After they have been trapped by the villous epithelium of the intermediate sac these aggregations of cells encounter a second system of free cells derived from the blood stream. This consists of lymphocytes, monocytes and polymorphonuclear cells (PMN). The luminal histocytes and PMN cells are degraded in the NVP in which are scattered monocytes and lymphoid cells. Free cells may also emanate from the epithelium of the intermediate sac. It is suggested that processing of antigen with the activation of lymphocytes occurs in the sac lumen and in the NVP. These cells together with macrophages containing concentrated digested material recirculate through the extensive network of lymph vessels surrounding this area of the ES. Some lymphocytes undergo differentiation into plasmoblasts and plasma cells.

ZUSAMMENFASSUNG

Die Sinnesorgane des Innenohres mit ihrer Nähe zu Infektionen ausgesetzten Bereichen sind wahrscheinlich abhängig von einer wirkungsvollen antimikrobiellen Verteidigung. In diesem Zusammenhang kann der sogenannte longitudinale Strom der Endolymphe in den Saccus endolymphaticus eine große Rolle spielen. Eindringende Stoffe können automatisch abtransportiert werden. Im distalen endolymphatischen Gang treten lymphoide Zellen und deutliche Phagozytose der Endolymphe auf. Im intermediären Abschnitt des Saccusepithels treten morphologische Anzeichen einer Modifizierung der einfließenden antigenen Substanzen zutage. Hier findet auch eine lebhafte Aktivität der Lymphozyten und Makrophagen miteinander statt, wie man sie auch in antigenaktivierten lymphoiden Geweben sehen kann. Hier befindet sich auch ein dichtes Netzwerk von Lymphkapillaren und Blutgefäßen.

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J Stahle M D
H Rask Andersen M D
ENT Department
University Hospital
S 750 14 Uppsal
Sweden

DISCUSSION

Albegger to Stahle Firstly I would like to congratulate you on the excellent morphological study and the interesting results In this connection I want only to stress two points

1 We have also found the same cell clusters in the human nasal and paranasal sinus mucosa, consisting of lymphocytes and macrophages But in contrast to your findings we could *not* detect any specialization of the cell membrane at the contact zones or even fusion of the cytoplasma at the sites of contact.

2 The second point concerns the nature of these cell clusters There is no doubt that such a cluster formation is common during the immunization process thus favoring intercellular cooperation which may be necessary in some kinds of immune reactions In subsequent experiments we found such cluster formations also in some other conditions without any immunization processes, for example in tissue cultures *Therefore we believe that the cluster formation may also be an unspecific and non-immunological phenomenon of immune-competent cells*

Thalmann to Stahl I would like to ask you about your estimate of bulk flow of endolymph towards the endolymphatic duct

Harrison to Stahle Has your material come from fresh human post-mortem specimens or the experimental animal On how many observations is your experience based?

Wersäll to Stahle The quality of the morphological study was very high. Have you analysed the quantity of active mononuclear cells by means of immunofluorescence in various parts of the labyrinth? Is there an even distribution or are they concentrated around the sac?

Surjdn Jr to Stahle It was interesting to see the accumulation of intraluminal cells Do you know what will happen with these cells

Jakobi to Stahle Following your results—is it too early to protect the inner ear by antibiotics in sterile operations, as in cases of otosclerosis

Rask-Andersen and Stahl (Reply)

Rask-Andersen to Albegger In our study we could not perceive direct cytoplasmic bridges between lymphocytes and macrophages such as were earlier demonstrated in various lymphatic tissues by Schoenberg et al This may be because the lymphoid cells in the sac are few in relation to lymphoid tissue and these direct intercellular communications are of short duration. We do not know whether the physical interactions between cells in the endolymphatic sac reflect specific immune reactions in progress in the sac but this matter should be investigated further

Rask Andersen to Thalmann No volumetric measurements have been performed but morphology may indicate a considerable resorption in the duct.

Rask-Andersen to Harrison We have examined almost 100 guinea pigs All the animals displayed non-vascularized epithelial processes but there were considerable variations in the number of freely floating cells in the sac

Rask Andersen to Wersäll We have performed immunofluorescence in order to identify the lymphocytes in the perisaccular tissue We found labelled cells in this tissue but also in the epithelium in the luminal space and especially in the NVP which suggests that a substantial number of the lymphocytes in the sac may belong to the B-lymphocyte population

Stahle to Portmann A deficient pressure regulation capacity of the duct and sac can be due to changes in the epithelium as well as in the vascular transport mechanism. Our finding of a rich network of lymph vessels surrounding the distal part of the endolymphatic duct may indicate that water reabsorption takes place mainly in this region. This specific function may be disturbed by periductal fibrosis or formation of new bone resulting in obstruction of the endolymphatic duct

Rask Anderse to Surjdn Jr The freely floating cells are degraded and phagocytosed by macrophages emanating from the perisaccular blood vessels as monocytes. The *cells concentrate in their cytoplasm indigestible cellular material which is most obviously seen when they recirculate through the lymph vessels*

Stahle to J kobi Our results indicate a built-in immuno-defence organ in the endolymphatic compartments. This might have shielded us from having labyrinthitis in childhood As far as otosclerosis surgery is concerned I never use prophylactic antibiotic treatment Our recent findings can hardly be used as an argument when discussing the value of prophylactic antibiotic treatment.

Acta Otolaryngol 89: 295–309 1980

OBLITERATION OF THE DUCTUS REUNIENS

Robert S Kimura, Harold F Schuknecht Carol Y Ota and Diane D Jones

From the Department of Otolaryngology, Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, Massachusetts, USA

Abstract The ductus reuniens was successfully obliterated in 57 guinea pig ears. Histopathological study showed that majority of these specimens demonstrated cochlear hydrops, saccular collapse and normal utricle. These results support the theory of longitudinal flow of endolymph from the cochlea toward the endolymphatic sac via the ductus reuniens and saccule. A major source of endolymph in the saccule appears to be the scala media. In another set of 11 animals in which the ductus reuniens was first obstructed and two months later the endolymphatic duct was blocked endolymphatic hydrops was shown in the cochleae, saccules, and utricles of all but one. The evidence suggests that cochlear hydrops was caused by obliteration of the ductus reuniens, and the saccular and utricular hydrops occurred subsequently as the result of blockage of the endolymphatic duct. Remnants of otolithic membrane which were attached to the distended saccular wall indicate that the membrane which had collapsed onto the macula after obliteration of the ductus reuniens is capable of subsequent distension. This experiment supports the concept of endolymph flow from the utricle and canals toward the endolymphatic sac. A blocked ductus reuniens might also explain the pathophysiological basis for the auditory form of Meniere's disease.

There are two principal theories concerning endolymph secretion and absorption in the membranous labyrinth. In the longitudinal flow theory endolymph of the cochlea is produced in the scala media and passes through the ductus reuniens saccule and endolymphatic duct to reach the endolymphatic sac where absorption takes place (Guild 1927 Lundquist 1965) Guild (1927) postulated that in the vestibular system there is a flow of endolymph from the utricle and ampullae through the utriculo-endolymphatic valve toward the endolymphatic sac. The radial flow theory contends that endolymph is both produced and absorbed locally within the scala media (Naftalin & Harrison 1958 Lawrence & Mc Cabe 1959 Lawrence et al. 1961 Lawrence 1966) as well as in the utricle (Dohlman 1964 1965) The radial flow theory appeared to gain support from reports by Lindsay (1947) and Suh & Cody (1977) that no change in membrane position occurred after obliteration of the endolymphatic sac in the monkey and chinchilla respectively On the other hand the occurrence of consistent endolymphatic hydrops in other species of mammals after obliteration of the endolymphatic duct or sac (Kimura & Schuknecht 1965 Kimura 1967 1976 Schuknecht et al 1968 Beal 1968 and others) supports the longitudinal flow theory

Since the results of these experiments are clearly in conflict an experiment was designed to further test the validity of the radial or longitudinal flow theory in the guinea pig which is ideally suited for this type of experiment The experiment consisted of morphological studies on two groups of guinea pigs one with obstruction of the ductus reuniens alone and the other with obstruction of the ductus reuniens followed later by obstruction of the endolymphatic duct. The findings might also have relevance concerning individual dependence of the auditory and vestibular labyrinths on the absorptive function of the endolymphatic sac Furthermore the experiment might shed light on the pathophysiology of atypical forms of Meniere s disease It is known that endolymphatic hydrops is the principal pathological feature of this disease and that most affected patients will exhibit both auditory and vestibular symptoms Atypical cases do occur however where the symptoms and functional test

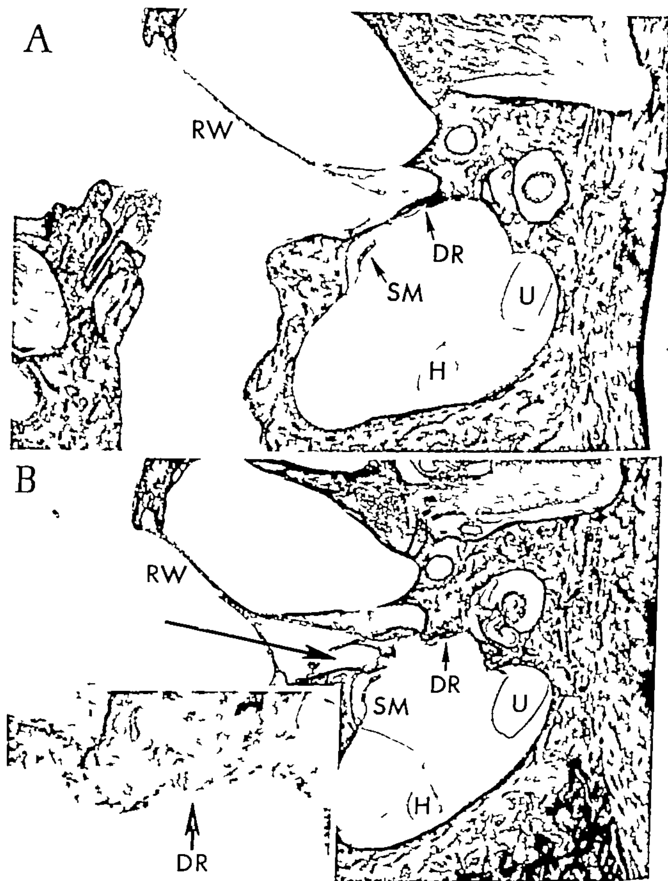


Fig 1 (A) The ductus reuniens (*DR*) is shown at the entrance of the scala media (*SM*) in a normal specimen. The ductus reuniens was followed to the saccule in serial sections. (B) The ductus reuniens was obliterated through the direction of the curved needle inserted to cut the ductus reuniens The inset shows a higher magnification of the obliterated ductus reuniens (*DR*) The same specimen is shown in Figs. and 3 *U* Utricle *H* horizontal canal

Table I

	Round window approach (35)	Vestibule approach (17)	Total (52)
A Cochlear hydrops, Saccular collapse, Utricle normal	23	12	35 (67%)
B Cochlear hydrops, Saccule normal, Utricle normal	6		8 (15%)
C Cochlear hydrops, Saccular collapse, Utricular hydrops		2	
D Cochlear hydrops, Saccular collapse, Utricular collapse	1	1	
E Cochlea normal, Saccular collapse, Utricle normal			2
F Cochlear collapse, Saccule normal, Utricle normal	1		1
G Cochlear collapse, Saccular collapse, Utricular collapse	2		2

deficits are either auditory or vestibular but not both

The experiment was based on the assumption that the reparative response to surgical interruption of the ductus reuniens would cause obstruction of its lumen. Previous experiments have shown the propensity of the labyrinth to heal surgically induced fistulae (Kimura & Schuknecht, 1975 Kimura et al. 1977) If a longitudinal flow does occur then obstructing the ductus reuniens should cause expansion of the scala media by accumulation of endolymph The saccule on the other hand, should collapse or remain normal If the endolymphatic duct were subsequently blocked the utricle ampullae and possibly the saccule would also become hydropic. If there were only a radial flow no change would be expected in the endolymphatic spaces In addition to the two sets of experiments appropriate control procedures were also performed The studies are concerned principally with the position of the membranous endolymphatic walls

MATERIALS AND METHODS

A total of 126 guinea pig ears were used for the entire experiment This large number was required since the surgical approach to the ductus reuniens was blind and surgical complications were involved In 89 ears only the ductus reuniens was obliterated by two different approaches round window (63) and vestibule (26) In 11 ears the endolymphatic duct was blocked two months after obliteration of the ductus reuniens by the round window approach In the remaining 26 ears three different control experiments were performed In many guinea pigs both ears were operated in order to conserve time space and effort

The operative approach to the ductus reuniens was through the external auditory canal. Under aseptic conditions the tympanic membrane was perforated and the cochlear duct was visualized through the round window membrane At the area close to the end of the cochlear duct a fine curved needle was inserted through the round window and basilar membranes and cuts were made by a scraping motion toward the osseous spiral lamina where the ductus reuniens enters into the cochlear duct. In 13 ears a stainless steel pin (465 μm) was pushed into the same area. The perforated round window membrane was covered with a small piece of gelfoam

In order to test the validity of the results obtained from the round window approach another surgical approach to the ductus reuniens was made. The bony wall posterior to the round window was drilled open and the ductus reuniens was reached through the cistern of the vestibule by a long curved needle Cuts were made at the predetermined area, at about the mid-length of the ductus reuniens The surgical procedure for obliteration of the endolymphatic duct and sac was described earlier (Kimura & Schuknecht, 1965)

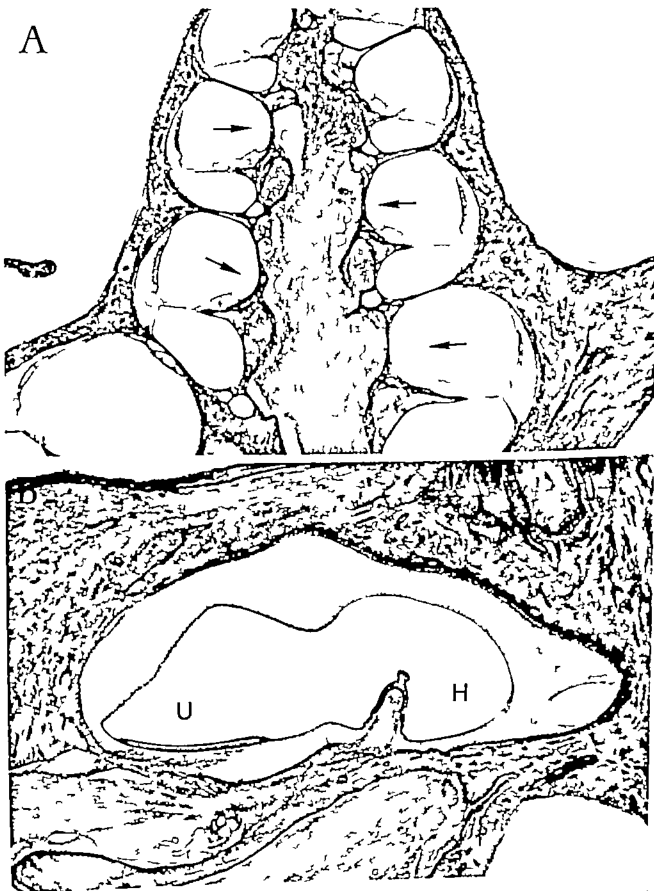


Fig 2 Both photographs are of the same specimen as shown in Fig 1B (A) The cochlea shows endolymphatic hydrops (arrows) resulting from obliteration of the ductus reuniens. (B) The utricle (*U*) and horizontal ampulla (*H*) remain normal

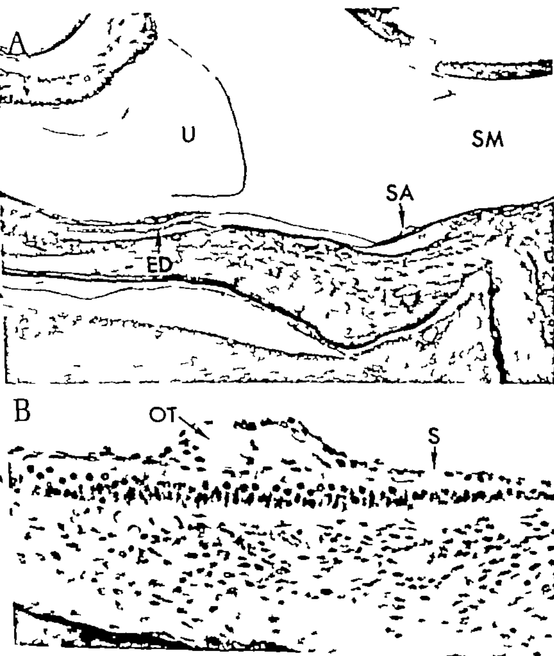
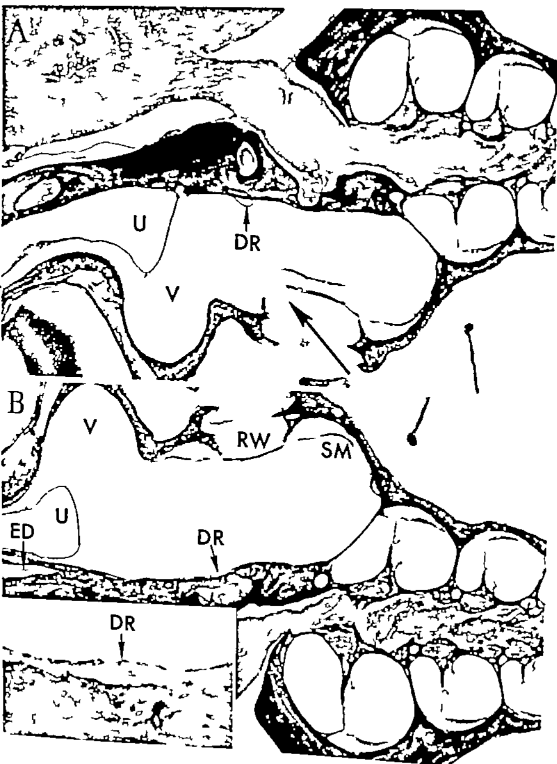


Fig. 3. The same specimen as shown in Figs 1B and 2. (A) The saccular membrane is collapsed after ductus reuniens obliteration. The distended scala media (*SM*) is shown at the right. The endolymphatic duct (*ED*) is normal and patent. *SA* Saccule; *U* utricle. (B) A higher magnification of the macula sacculi in Fig. 3A showing the degenerated otolithic membrane (*OT*). The mass is surrounded by the saccular membrane (*S*).

As controls of the experiment, the round windows were opened in six ears, the cochlear ducts were perforated through both Reissner's and basilar membranes in 13 ears and stapedes were mobilized or subluxated in seven ears. The saccular perforations performed in nine ears of an earlier experiment (Kimura et al. 1977) served as another experimental con-

Fig 2 Both photographs are of the same specimen as shown in Fig. 1B (A) The cochlea shows endolymphatic hydrops (arrows) resulting from obliteration of the ductus reuniens. (B) The utricle (*U*) and horizontal ampulla (*H*) remain normal


A
U
DR
V
B
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ED
DR
DR

	Hydrops	Collapse	Normal
Round Window approach (35)			
Cochlea	30 (86%)	3	2
Saccule	0	28 (80%)	7
Utricle	0	7	28 (80%)
Vestibule approach (17)			
Cochlea	17 (100%)	0	0
Saccule	0	15 (88%)	2
Utricle		1	14 (82%)
Total (52)			
Cochlea	47 (90%)	3	
Saccule	0	43 (83%)	9
Utricle	2	8	42 (81%)

trol In addition 80 other normal or unoperated ears were studied as controls

The survival times of almost all the experimental animals in the present study were five months In animals in which two operations were performed the survival time was five months after ductus reuniens obliteration and three months after obliteration of the endolymphatic duct All animals were systemically perfused with Heidenhain-Susa fixative and the ears were processed by the routine celloidin technique for serial sectioning at 20 μ thickness Every tenth section was stained and examined In many specimens all serial sections were examined in the area where the ductus reuniens had been surgically obliterated

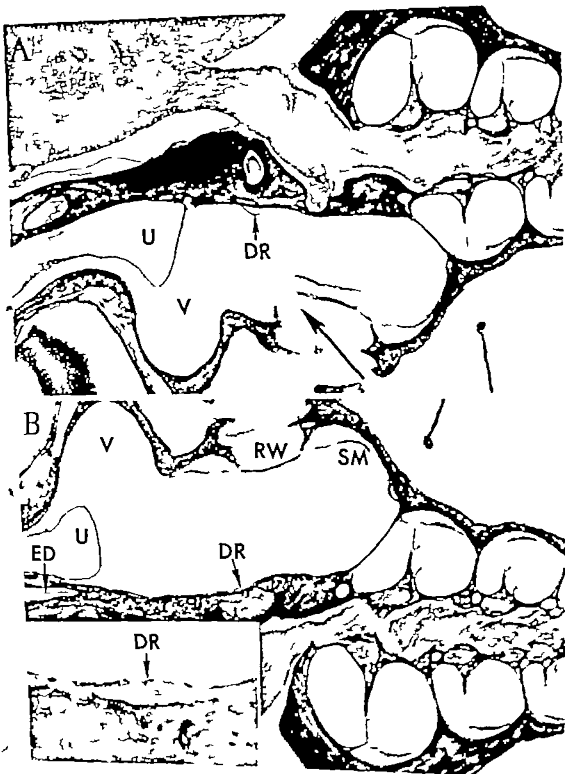
The histological appearance of the ductus reuniens was evaluated at the initial segment where trauma was introduced the area adjacent to the trauma, the mid point of the duct and the area close to the saccule The ductus reuniens was judged as being completely absent (or atrophied) collapsed or normal The evaluation was made without prior knowledge of the pathological alterations in other parts of the membranous labyrinth In another separate evaluation the positions of the membranous walls of the cochlear and vestibular labyrinths were recorded without prior knowledge of the condition of the ductus reuniens These two sets of data were put together for same procedures were repeated in other normal and control experimental specimens

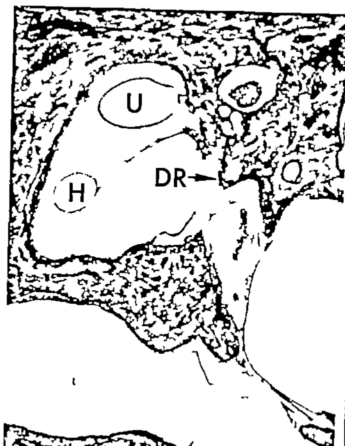
FINDINGS

Although 89 ears were operated to interrupt the ductus reuniens histological studies revealed successful obliteration in only 52 (Fig 1) The remainder showed no obliteration partial damage or reconnection of the membranous duct or they demonstrated otitis media and/or inflammatory reaction concomitant with obliteration of the ductus reuniens When the positions of the membranous walls of the 52 inner ears were studied several different combinations of features became apparent (Table I) The most frequent combination was cochlear hydrops saccular collapse and normal utricle which occurred in 35 out of 52 ears (67%) (Figs 2 and 3) The second most frequent combination was cochlear hydrops with normal saccule and normal utricle (8 of 52 15%) When the positions of the membranous walls in each structure were compared the cochlea showed the highest incidence of endolymphatic hydrops (90%) the saccule showed the highest incidence of collapse (83%) and the utricle was most frequently normal (81%) (Table II)

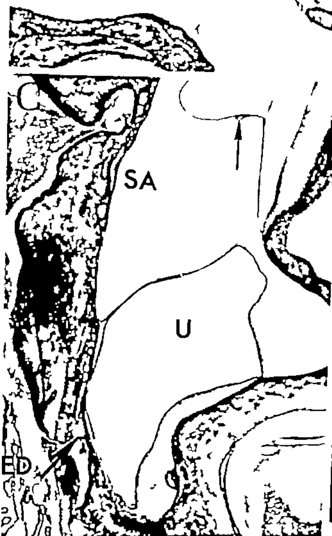
The approach to the ductus reuniens via the vestibule was more successful than via the round window Insertion of a pin into the cochlear duct near the ductus reuniens through the round window did not improve the rate of successful obliteration about 50% of 13 ears Although the approach through the

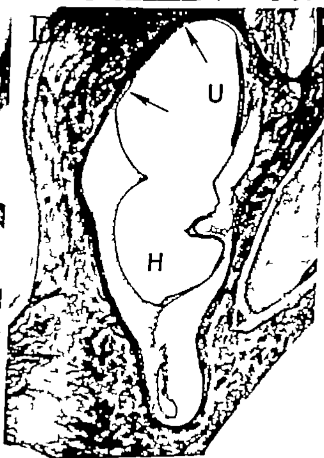
Fig 4 (A) The ductus reuniens (*DR*) is shown at a location close to the saccule The lumen is open in spite of the fact that the scala media was perforated through the round window membrane (arrow) as a control Note the normal position of Reissner s membrane *U* Utricle V vestibule (B) The ductus reuniens (*DR*) was obliterated by the approach from the vestibule The operative site is not shown A higher magnification of the obliterated ductus reuniens (*DR*) is shown in the inset. Note the cochlear hydrops. *ED* Endolymphatic duct *U* utricle V vestibule *RW* round window *SM* scala media.


A
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DR


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H

vestibule was a more certain method for obliterating the ductus reuniens (Fig 4) the macula sacculi was often traumatized Opening the bony capsule produced inflammatory reactions of fibrosis and ossification at the operative site and adjacent perilymphatic space In some specimens the lumen of the endolymphatic duct became very narrow or closed or the utriculo-endolymphatic valve appeared to be closed both of which occurred in association with endolymphatic hydrops of the utricle

Histopathological alterations in the cochlea resulting from the round window approach were atrophy of sensory cells spiral ganglia and striae vasculares in the basal and apical turns. The connective cells of the limbus often degenerated in corresponding areas The sensory cells of the saccule often became atrophic particularly in the areas where the saccular membrane had collapsed on top of the degenerated otolithic membrane It was common to see small remnants of the otolithic membrane encapsulated by cells which were similar to the cells of the saccular membrane (Fig 3B) The utricles were normal The ampullae were normal except for a few atrophic posterior cristae, possibly caused by trauma associated with inadvertent surgical perforation of the adjacent utricular wall

Histopathological changes observed in specimens with the vestibular approach were similar to those of the round window approach although more severe sensory cell changes were seen at the extreme basal end of the cochlea where drilling occurred or where the spiral ligament was sometimes traumatized The saccules showed degenerated neuroepithelium often as the result of direct trauma. The utricles and most of the ampullae remained normal There were atrophic changes in some of the ampullae of the posterior semicircular canals

In almost all 80 normal control ears the ductus reunientes remained open They were collapsed in a few specimens but were not obliterated or atrophic as observed in experimental ears The positions of the membranous walls of the cochleae saccules and utricles remained normal in a majority of the specimens A slight distension of Reissner s membranes in the round window areas or a slight collapse of the utricles was sometimes noted One specimen showed a diffuse collapse of the cochlea, saccule and utricle

In the 13 control ears in which the round window membranes and cochlear ducts were pierced by a needle the ductus reunientes remained open in 12 all of which showed normal position of Reissner's membranes. One specimen showed an obliterated ductus reuniens due to accidental trauma which was associated with a slight distension of Reissner s membrane Most of the saccules and utricles remained normal though a few showed slight collapse In 11 experimental ears in which obliteration of the ductus reunientes failed the positions of the membranous walls of the cochleae saccules and utricles remained normal In the six control ears in which the round window membranes were opened and seven ears in which stapedes were mobilized or subluxated the ductus reunientes remained open with one exception of collapse in the stapes mobilization series The positions of the membranous walls of the inner ears remained normal with the exception of collapse in the utricles In the nine ears undergoing sacculotomy the ductus reunientes were all open. The positions of the membranous walls were normal with the exception of one which showed hydrops in the cochlea, saccule and utricle and blockage of the endolymphatic duct presumably due to surgical complication

Fig 3 In this specimen the ductus reuniens was obliterated and two months later the endolymphatic duct was blocked. (A) The ductus reuniens (*DR*) was obliterated through the round window membrane. *U* Utricle *H* horizontal canal (B) The cochlea shows endolymphatic hydrops. (C) The endolymphatic duct (*ED*) is obliterated and the saccule (*SA*) shows hydrops. Arrow points to the remnant of degenerated otolithic membrane attached to the distended saccular membrane A higher magnification is shown in Fig. 6C. *U* Utricle (D) The utricle (*U*) shows hydrops (arrows). Collapse of the horizontal canal near its ampulla (*H*) is a specimen preparation artifact

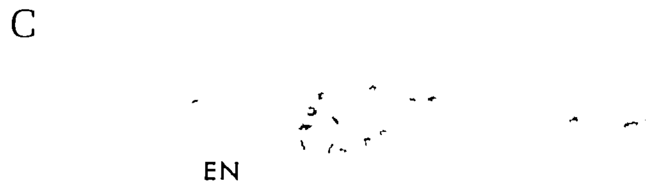
A

ST

OT

SA





In the series of 11 animals in which the endolymphatic duct was blocked two months after surgical obliteration of the ductus reuniens through the round window hydrops was observed in all cochleae saccules and in 10 utricles (Fig. 5) The magnitude of distension of these membranes was similar to that which follows blockage of the endolymphatic duct alone In 8 of 11 ears the distended walls of the saccules contained small remnants of the atrophic otolithic membranes structures similar to those observed on the collapsed saccular maculae in the series undergoing blockage of the ductus reunientes (Fig. 6) Other histopathological changes were neurosensory lesions at the basal ends or in both basal and apical turns similar to those seen in the ductus reuniens series Two specimens showed sensory cell atrophy in small areas of the saccular maculae. The sensory cells of the utricular maculae and cristae were normal except for for three posterior cristae which showed degeneration.

DISCUSSION

It might be argued that all these pathological alterations could be the result of a non-bacterial traumatic labyrinthitis and therefore are not related to obliteration of the ductus reuniens. Of course all invasive surgical procedures produce inflammatory and reparative responses One method of experimental control is to perform another invasive procedure which is similar but selectively different from the one under study When such controls were performed (opening of the round window perforation of the scala media, stapes mobilization) the resultant histopathological features were significantly different from those following obliteration of the ductus reuniens Even in our simple sacculotomy series (Kimura et al 1977) results were unlike those of the present ductus reuniens series Thus histopathologic observations in the majority of the specimens appear to be related to obliteration of the ductus reuniens Various other histopathological features in a small number of specimens could be attributed to labyrinthitis specimen preparation artifact tears in other parts of the membranous wall or simply non-healing of the fistulized membranes

The main histological characteristic after obliteration of the ductus reuniens was the combination of cochlear hydrops saccular collapse and normal utricle The combination with a much lower incidence was cochlear hydrops and normal saccule and utricle The cochlear hydrops may be explained if we presume that endolymph normally flows toward the ductus reuniens saccule and endolymphatic sac. If the ductus reuniens were blocked and endolymph were still being produced then Reissner's membrane would distend Supporting evidence from another experiment is the demonstration of cochlear hydrops after blockage of the endolymphatic duct, as stated earlier In a labyrinthotomy experiment in which the hook region of the cochlea was surgically destroyed the remaining parts of the cochlea developed hydrops (Hoshino & Paparella, 1970) Gussen (1978) reported that atrophy of the human macula sacculi resulted in accumulation of otoliths or otolithic membrane debris in the ductus reuniens and/or in the cochlear duct The tissue reaction at these areas caused development of hydrops in the basal turn and even throughout the cochlea.

The saccule in the present study collapsed possibly because it was deprived of its main source of endolymph from the scala media. There are no secretory cells (dark cells) in the saccule (Kimura, 1969) A close physiological relationship between the cochlea and saccule was also shown by the collapsed Reissner's

Fig 6 The saccular membranes of specimens in which the ductus reuniens was obliterated first followed by blockage of the endolymphatic duct two months later (A) The saccular membrane with the remnant of the otolithic membrane (*OT*) is partly lifted in the hydropic saccule (*SA*). *ST* Stapes footplate (B) The remnant of the otolithic membrane is attached to the distended saccular membrane. The same specimen as shown in Fig. 5C *EN* Endolymph side (C) Another specimen showing the lifted degenerated otolithic membrane attached to the distended saccular membrane *EN* Endolymph side

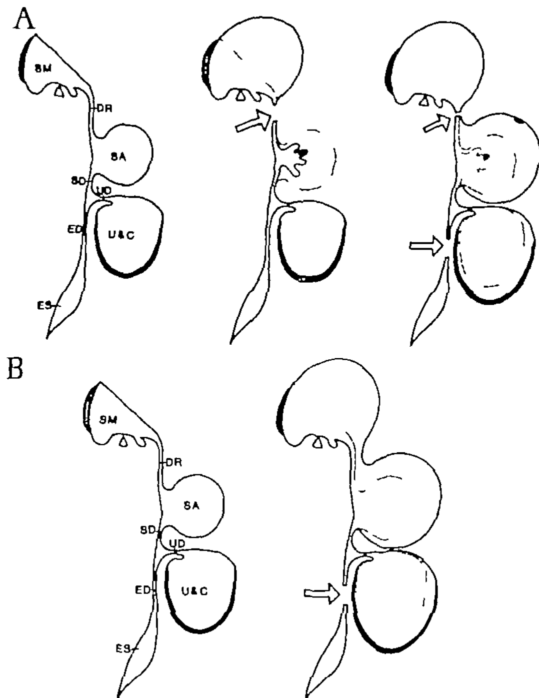


Fig 7 Drawings summarizing present and past experimental observations. Drawings on the *left side in both* figures represent the normal positions of the membranes *SM* Scala media, *DR* ductus reuniens *SA* saccule *SD* saccular duct *UD* utricular duct *U & C* utricle and canals *ED* endolymphatic duct *ES* endolymphatic sac. The cross-shaded areas represent the stria vascularis and dark cells. Note absence of dark cells in the saccule (A) Changes after obliteration of the ductus reuniens show cochlear hydrops saccular collapse with degeneration of *the otolithic membrane (black dot) and normal utricle* (middle drawing) When the endolymphatic duct is blocked two months later the saccular membrane distends more than the original normal position and carries the remnant of the otolithic membrane with it The utricle becomes hydropic (right drawing) (B) The position of the membranous endolymphatic walls after blockage of the endolymphatic duct only

and saccular membranes in hereditarily deaf dogs (Lurie 1948 Schuknecht et al 1965 Mair 1976) *in white mink* (Sugiura & Hilding 1970) and in white cats (Bosher & Hallpike 1965 Mair 1973) though the utricular wall remained normal When endolymph was withdrawn from the scala media Reissner s and saccular membranes collapsed whereas the

utricle remained unaltered (Bast & Anson 1947) In the present study the collapse of the saccular wall seen in the round window approach is not caused by direct trauma of the saccule by the needle nor is it likely to be due to failure of the severed ductus reuniens to heal In one control specimen of the scala media perforation series the basilar membrane failed to heal but the ductus reuniens remained open and saccular and Reissner s membranes remained in normal position The normal position of the saccular membrane in the presence of cochlear hydrops in a small number of specimens may suggest either that endolymph may also come from the utricle or it may come from the scala media as a result of incomplete closure of the ductus reuniens. Even in the genetically involved inner ears previously mentioned the saccules close to the side of the utricles and endolymphatic sacs were open The utricles remained essentially normal since the mechanism for fluid secretion and absorption was not altered Some utricular collapses could be due to specimen preparation artifact since similar conditions are occasionally seen in normal specimens.

When obliteration of the ductus reuniens was followed by blockage of the endolymphatic duct two months later all cochleae saccules and utricles (except one) showed endolymphatic hydrops Cochlear hydrops may have been caused by either obliteration of the ductus reuniens or blockage of the endolymphatic duct if the ductus reuniens were not obliterated earlier The saccular hydrops may be explained either by endolymph coming from the scala media following blockage of the endolymphatic duct in situations when the ductus reuniens was not obliterated or by endolymph coming from the utricle via the utriculo-endolymphatic valve utricular duct and saccular duct when the ductus reuniens was obliterated. The second possibility is very much in evidence in that the degenerated otolithic membrane was observed attached to the distended saccular membrane As previously shown, when the ductus reuniens is obliterated the saccular membrane collapses In the collapsed stage the membrane on top of the macula surrounds the degenerated otolithic membrane but it is evidently not firmly attached to the macula. When the endolymphatic duct is blocked the collapsed membrane with the otolithic remnant is raised and keeps stretching beyond the normal position due to accumulation of endolymph which presumably comes from the utricular side (Fig. 7) Perhaps this evidence may also serve as indirect support for the concept of endolymph drainage from the utricle and canal organs toward the endolymphatic sac as postulated by Guild (1927) It is at odds with the opinion expressed by Seymour (1959) that the fluid from the endolymphatic sac serves to insure complete filling of the utricle and canal endolymphatic spaces

The present experiment upholds the theory of longitudinal flow of endolymph from the cochlea, saccule utricle and canals toward the endolymphatic sac. In spite of the fact that the radial flow theory is not supported by this study it does not mean of course that such a flow could not also be present. Also there is no proof that endolymph is solely absorbed at the endolymphatic sac Particles injected into the perilymphatic space appear in the scala media and eventually in the stria vascularis (Hinojosa 1972) or in the outer sulcus cells and spiral prominence cells (Altmann & Waltner 1947) and a dye injected into the canal endolymph is absorbed by the dark cells (Dohlman 1964) The localized disturbance in fluid metabolism or changes in membrane characteristics could cause a localized hydrops or collapse as shown in the present study Although this interpretation is plausible at present there is no strong or direct experimental evidence to support this concept

The demonstration of endolymphatic hydrops limited to the cochlea following obstruction of the ductus reuniens offers a pathophysiological explanation for the auditory type of Meniere s disease While most patients with Meniere s disease (idiopathic endolymphatic

hydrops) exhibit both hearing loss and vertigo a small number show only hearing loss The features of the hearing loss are distinctive and are characterized by fluctuations in thresholds and dominant low tone loss early in the dis ease Lindsay & von Schulthess (1958) have reported the only human temporal bone study in which endolymphatic hydrops was limited to the cochlea Unfortunately they made no comment as to the condition of the ductus reuniens in this case

ACKNOWLEDGEMENTS

The authors are grateful to Barbara Burgess and Richard Cortese for their technical assistance

This work was supported by NINCDS Grant no 5 R01 NSO 3932 18

ZUSAMMENFASSUNG

Der Ductus reuniens wurde in 52 Meerschweinchen er folgreich obliteriert Die histopathologische Untersuchung zeigte daß die Majorität der Präparate cochlearen Hy drops Kollaps des Sacculus und einen normalen Utri culus aufwies Diese Resultate unterstützen die Theorie eines longitudinalen Endolymphflusses von der Cochlea zum Saccus endolymphaticus via Ductus reuniens und Sacculus Eine Hauptquelle von Endolymphe scheint die Scala media zu sein In 11 Meerschweinchen bei denen der Ductus reuniens unterbrochen wurde und erst zwei Monate später der Ductus endolymphaticus blockiert wurde wurde endolymphatischer Hydrops in allen Coch leae Sacculi und Utriculi (außer einem) demonstriert Die Evidenz läßt vermuten, daß Hydrops der Cochlea durch Obliteration des Ductus reuniens, während Hydrops des Sacculus und Utriculus durch Blockieren des Ductus endolymphaticus verursacht werden Reste der Otolithen-Membran die an der gedehnten Membran des Sacculus hafteten zeigten daß die Membran des Sacculus die uber der Macula kollabiert war nach Obliteration des Ductus reuniens gedehnt worden war Dieses Experiment unterstützt das Konzept eines Endolymphflusses vom Utriculus und den Kanälen zum Saccus endolymphaticus. Ein blockierter Ductus reuniens könnte pathophysiolo gisch die auditorische Form des Morbus Meniere er klären

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Robert S Kimura Ph.D
Department of Otolaryngology
Massachusetts E and Ear Infirmary
43 Charles Street
Boston Massachusetts 02114 USA

DISCUSSION

Wersäll to Kimura I have always admired Dr Kimura xperimental surgical ability I want to congratulate you on the quality and consistency of your studies I find your present work very important for the understanding of endolymph circulation I have only one question. W know now that the dark cells are only found in the areas in the vestibular part of the labyrinth where we have endolymph production. Can you speculate on the function of the dark cells

Spoendlin to Kimura What is the size or rapidity of endolymph flow toward the endolymphatic sac? The second question is what is the surgical approach to the ductus reuniens through the estibule?

Sadé to Kimura Your excellent experiments leave little doubt that there is an endolymphatic flow from the cochlea to the endolymphatic duct and sac, and that blocking of this pathway results in distention (hydrops) proximal to the obstruction However as surgeon I must ask myself as to the functional meaning of the endolymphatic duct (or sac) obstruction Because I (like other ear surgeons) have seen and published cases where slow bone resorption associated with deep cholesteatoma has destroyed the entire labyrinth—at times not sparing the vestibule wall (sparing only the cochlea), while the hearing before as after the operation showed normal or near-normal bone conduction. In these ears the endolymphatic duct as obviously included in the destructive process. If such ears developed histological endolymphatic distention—the so-called "hydrops"—then this hydrop caused them little harm. This tallies ell with the observation that such endolymphatic distention as found in Meniere patients is also found in multitude of other ear diseases or indeed at times in ears with no history of deafness or vertigo.

It would therefore seem that narrowing or closing of the endolymphatic duct and sac might be less dangerous than is usually assumed—and that the histological picture of a dilated endolymphatic ystem is unspecific and possibly does not alw ys signify pathological process

The connotations of the above to the various treatments applied to patients suffering from vertigo are self-evident.

Thalmann to Kimura I am particularly impressed by the collapse of the saccule since this is further proof of the secretory role of the dark cells which according to your previous studies are lacking in the saccule Also the resting potential of the saccule has been show to be merely remote manifestation of the endocochlear potential Your xperimental pathological results agree beautifully w th current electrophysiological concepts about the role of the saccule.

Kimura (Reply)

T Wersäll I am very grateful for you nice compliment. At present I cannot give you more information on the function of dark cells I am sure that future study will reveal more specific function of the dark cells.

T Spoendlin It is my impression that the size of the endolymph flow toward the endolymphatic sac is rather small and slow This opinion is based on my experience of opening the normal endolymphatic sac However it should be noted that hydrops develops in the cochlea and saccule within 24 hours after obliteration of the endolymphatic duct. The surgical approach to the ductus reuniens through the vestibule is to open the bony wall posterior to the round window This is accomplished by thinning the bone with burr and deep circular groove is made with a pointed instrument. The bony disc and fragments are lifted out with fine hook. A long curved needle is inserted to cut the ductus reuniens of which the location was predetermined by the previous anatomical study

T Sadé I do not have an answer to your clinical observations. The histopathological study related to the present investigation is case of hydrops limited to the cochlea ithout having the typical symptoms of Menie re disease as reported by Lindsay and von Schulthess (1958) Gussen reported last year that cochlear hydrops was noted in the cases of cochlear and saccular degeneration. She attributed hydrops to the degenerated otolithic membrane entering into the ductus reuniens and the cecum vestibulare where fibrosis and inflammatory reaction developed.

T Thalmann. Collapse of the saccule membrane is a real phenomenon. I am still convinced that there are no dark cells in the saccule The main source of endolymph is probably the scala media. Distribution of dark cells in the vestibular labyrinth is mapped in my previous publication. They are shown in all three ampullae and utricle. A part of endolymph in the saccule may come from dark cells of the utricle and ampullae



Acta Otolaryngol 89 310–316 1980

ZUM COGAN SYNDROM

G Zechner

Aus der HNO-Abteilung des stadtischen Krankenhauses Wien-Lainz, Osterreich

Abstract Cogan s Syndrom is the clinical entity of non syphilitic interstitial keratitis with vestibuloauditory symptoms. Although the disease is not rare the pathohistological findings in temporal bones are only reported twice Our communication deals with observations made on a temporal bone out of O Mayer s collection The most striking findings are the endolymphatic hydrops the degeneration of the organ of Corti the pathology of the ductus reuniens the fibrosis within the cisterna perilymphatica, the destruction of the maculae and the most uncommon ossification within the scala vestibuli of the basal turn. The vessels seem to show signes of angiitis

Das Cogan Syndrom wurde erstmals 1945 als *nichtsyphilitisch interstitielle Keratitis* mit audiovestibulären Symptomen vergesellschaftet beschrieben Seither wurde über etwa 60 Fälle dieser Erkrankung unklarer Ätiologie berichtet *zuletzt 1976 durch Edström und* Vahlne Allerdings gibt es nur von zwei Patienten *pathohistologische Befunde von Fel*senbeinen einmal 1961 von Fisher und Hellström *und 1965 von Wolff Bernhard e a* veröffentlicht. Aus der Sammlung O Mayers *war es mir möglich* die Schnittserie eines Felsenbeines zu untersuchen die die Kriterien der perilymphatischen Fibrose zeigen Auffallend war daß O Mayer den Befund als sehr bemerkenswert eingestuft hatte ihn aber nicht ausgedeutet hatte Der Patient ist 1921 an allgemeiner Arteriosklerose gestorben und war 54 Jahre alt. Der Befund wurde von Gimplinger verwendet als er 1926 eine Abhandlung über Knochenneubildung im Labyrinth veröffentlicht hatte

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Eigene Befunde und Vergleich mit den Beobachtungen von Fisher und Hellström sowie Wolff und Mitarbeiter

1 Trommelfell etwas verdickt durch starke Vascularisation die Faserstruktur etwas undeutlich jedoch keinerlei Entzündungsresiduen Keine Perforation Im äußeren Gehörgang soweit erhalten Haut und Anhangsdrüsen unauffällig wie auch bei Fischer und Hellström

2 Mittelohr Im Lumen kein pathologisches Sekret *zarte Schleimhaut ohne* Entzündung oder Narben Die ovale Fenster*nische frei ohne* Sekretretention Der Steigbügel samt Muskel unauffällig Der Kanal zum runden Fenster frei die Membran zart Wolff u a beschreiben neugebildeten Knochen

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Herrn Univ Prof Dr E H Majer zum 70 Geburtstag in Dankbarkeit gewidmet.
Mit Unterstützung des Fonds zur Förderung der Wissenschaftlichen Forschung in Österreich.



Abb 1 HE gefärbt Gefäßbündel entlang des Nervus facialis

innerhalb der verdickten Membran Um den Gesichtsnerv gelagerte Gefäße am besten zu sehen nahe der ovalen Fenstergegend zeigen Veränderungen. Die Venen sind sehr weit und teilweise thrombosiert, die Arterien mit ver dickter Wand haben eine homogenisierte Media und herdförmig verquollene Intima (Abb 1)

3 Schnecke. Wie im gesamten häutigen Labyrinth finden sich reichlich Pigmentablagerungen in den bindegewebigen Anteilen der Kanalwände Am auffälligsten ist die starke Ausweitung des Ductus cochlearis in allen drei Windungen am stärksten in der Gegend des Helicotrema, durch welches eine richtige Her niation erfolgt. In der Basalwindung liegt in der Scala vestibuli lockeres areoläres Binde gewebe In dieses eingebettet hyaline Schollen und der Labyrinthkapsel anliegend Herde neugebildeten Knochens (Abb 2) In der Mittel und Spitzenwindung hingegen ist die Scala vestibuli aufgebraucht, die Reißner sche Membran liegt praktisch dem Modiolus an. Eine hernienartige Ausstülpung der zer dehnten Reißner'schen Membran in die mit Bindegewebe aufgefüllte Scala vestibuli ist



Abb 2 HE gefärbt, Basalwindung der Cochlea mit Fibrose Knochenneubildung in der Scala vestibuli

Acta Otolaryngol 89 310–316 1980

ZUM COGAN-SYNDROM

G Zechner

Aus de HNO-Abteilung des stadtischen Krankenhauses Wien-Lain., Österreich

Abstract Cogan s Syndrom is the clinical entity of non syphilitic interstitial keratitis with vestibuloauditory symptoms. Although the disease is not rare the pathohistological findings in temporal bones are only reported twice Our communication deals with observations made on a temporal bone out of O Mayer's collection The most striking findings are the endolymphatic hydrops the degeneration of the organ of Corti the pathology of the ductus reuniens the fibrosis within the cisterna perilymphatica, the destruction of the maculae and the most uncommon ossification within the scala vestibuli of the basal turn The vessels seem to show signes of angitis

Das Cogan Syndrom wurde erstmals 1945 als nichtsyphilitisch interstitielle Keratitis mit audiovestibulären Symptomen vergesellschaftet beschrieben Seither wurde über etwa 60 Fälle dieser Erkrankung unklarer Ätiologie berichtet zuletzt 1976 durch Edström und Vahlne Allerdings gibt es nur von zwei Patienten pathohistologische Befunde von Felsenbeinen einmal 1961 von Fisher und Hellström und 1965 von Wolff Bernhard e a veröffentlicht Aus der Sammlung O Mayers war es mir möglich die Schnittserie eines Felsenbeines zu untersuchen die die Kriterien der perilymphatischen Fibrose zeigen Auffallend war daß O Mayer den Befund als sehr bemerkenswert eingestuft hatte ihn aber nicht ausgedeutet hatte Der Patient ist 1921 an allgemeiner Arteriosklerose gestorben und war 54 Jahre alt Der Befund wurde von Gimplinger verwendet, als er 1926 eine Abhandlung über Knochenneubildung im Labyrinth veröffentlicht hatte

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Unseren Befunden nach ist sie ein Teil der herdförmigen perilymphatischen Fibrosierung die beherrschendes morphologisches Substrat dieser Erkrankung ist

4 Die Veränderungen an der Stria vascularis möchten wir nicht wie Wolff und Mitarbeiter als Hauptursache des Hydrops anführen Sie könnten sicher beitragen durch vermehrte Lymphproduction was aber bis heute nicht beweisbar ist

Unsere morphologischen Befunde erlauben aber auch Rückschlüsse auf die Ätiologie der Erkrankung Da unser Fall allerdings kein akuter war können wir zu den sehr bemerkenswerten Befunden erhoben von Edström und Vahlne nicht Stellung nehmen. Wenn wir allerdings die Beobachtungen an den Gefäßen im Felsenbein als panarteriitis-nodosa ähnlich festlegen so wird die Störung im Immunsystem, über welche sie als Infektionsfolge berichten sehr wichtig. Auch unser Patient erlitt den ersten Schub des Hörverlustes im Rahmen einer Infektion Ob und wie eine Autoimmunerkrankung daraus enstanden ist können wir mangels Befunden an anderen Organen nicht festlegen Aber die Veränderungen an den Gefäßen rund um den Nervus facialis und an der Arteria labyrinthi sowie die Thrombosen in den Venen der Bogengangswand und der Begleitvene des Aquaeductus vestibuli können als pathohistologischer Beweis gelten. Wir möchten deshalb Oliner und Mitarbeiter sowie Fisher und Hellström unterstützen und auch Cogan und Dickersen, die annehmen daß das Cogan Syndrom eine Teilform der Panarteriitis nodosa ist, ähnlich der Wegener'schen Granulomatose in unserem Fachgebiet Somit wäre das Syndrom den Autoimmunerkrankungen zuzurechnen, wofür auch die Therapieerfolge mit hochdosiertem Cortison berichtet von Smith 1970 sprechen würden.

ZUSAMMENFASSUNG

Es wird über pathohistologische Befunde erhoben an einem menschlichen Felsenbein beim Cogan Syndrom berichtet. Die hervorstechenden Beobachtungen sind: 1 Fibrose im perilymphatischen Raum Scala vestibuli cysterna perilymphatica vestibuli rund um den oberen Bogengang und den Ductus reuniens. 2. Ossificationen in perilymphatisch-fibrosierten Arealen: Scala vestibuli der basalen Schneckenwindung. Vestibulum. 3 Zerstörung der Sinnesendstellen: Corti'sches Organ macula sacculi und utriculi 4 Endolymphatischer Hydrops mittlere, obere Schneckenwindung, Sacculus, Utriculus oberer Bogengang. 5 Perisacculäre Fibrose im Ductus endolymphaticus und Blockade der Bast'schen Klappe 6. Panarteriitis nodosa ähnliche Gefäßveränderungen an der Arteria labyrinthi den Venen um den Aquaeductus vestibuli und an allen Gefäßen um den Nervus facialis. Auf Grund dieser Veränderungen nehmen wir an daß es sich beim Cogan Syndrom um eine localisierte Erkrankung aus dem Formenkreis der Autoimmunerkrankungen handelt.

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Prof dr G Zechner
Hals Nasen- Ohrenheilk.
Wolkersbergen str 1
A-1130 Wien
Austria

DISCUSSION

Kelemen to Zechner: Remarks of birth of Cogan syndrome at the Massachusetts Eye & Ear Infirmary until its knowledge grew as shown in the masterful presentation.

u a. erwähnt Auch konnten wir keine blauen Mäntel in der Labyrinthkapsel erkennen

5 Der Aquaeductus vestibuli zeigt eine Fülle von Veränderungen Die Bast sche Klappe am Eingang ist durch perilymphatische Bindegewebswucherungen verzogen kaum erkennbar Das perisacculäre Gewebe ist stark fibrotisch das Lumen eingeengt dort wo er halten mit Kolloidkugeln ausgefüllt

Das duranahe Ende ist extrem schlitz förmig Die Befunde an den Begleitgefäßen erscheinen uns sehr wichtig Die Vene ist thrombosiert und die Arterien um die Pars rugosa fehlen fast vollkommen Dies leitet über zur Arteria labyrinthi welche wir im Porus acusticus internus mit stark verdickter Wand sklerotischen Plaques und deutlicher Intimaverquellung beobachten konnten Eine entzündliche Reaktion perineural haben wir nicht gesehen Spärlich zellige Infiltrationen scheinen nur perivasculär zu liegen

Besprechung der Befunde Die erhobenen Befunde sprechen für eine perilymphatische Fibrose wie sie kennzeichnend ist für das Cogan Syndrom Neben der Fibrose besteht herdförmige Knochenbildung in der Scala vestibuli und in der perilymphatischen Cyster ne des Vorhofs Die Ektasien des Endolymph-schlauches fanden wir am stärksten ausge prägt im Ductus cochlearis der mittleren und oberen Schneckenwindung im Sacculus mehr als im Utriculus aber auch im oberen Bogen gang Daraus glauben wir zwei Dinge ableiten zu können

1 Die Erkrankung befällt das Innenohr herdförmig

2 Die Gefäße scheinen eine wichtige Rolle zu spielen was ja auch schon Fisher und Hellström Cody und Williams u a mehr angenommen haben

Die von uns erhobenen Gefäßbefunde haben einerseits große Ähnlichkeit mit panar teritis nodosa ähnlichen Veränderungen aber auch starker Arteriosklerose Laut Obduk tionsprotokoll ist dies auch die Todesursache des Patienten

Die Erkrankung muß in Schüben verlaufen

Alls morphologische Belege können wir an führen

1 Die hernienartige Ausbuchtung der Reiß ner schen Membran in die von Bindegewebe bereits ausgefüllte Scala vestibuli der basalen Schneckenwindung

2 Der unregelmäßig zerdehnte Sacculus – Utriculus und Bogengangsapparat allerdings anders als beim typischen Endolymphhy-drops Dies rückt eine Annahme Arslans in ein anderes Licht ,Bei der Ausweitung des Endolymphschlauches spiele der Perilymph-raum nicht nur eine passive Rolle In un-serem Falle scheint das perilymphatische Bindegewebe einen deutlichen Zug auf die Wände des häutigen Labyrinths auszuüben

Vom sogenannten idiopathischen Endo lymphhydrops grenzt sich die Erkrankung durch folgende morphologische Details ab

1 Die Sinnesendstellen sind insbesondere in der Schnecke stark pathologisch verändert.

2 Die Stria vascularis zeigt neben Colloid-zysten Kalkeinlagerung auch gefäßbezoge ne Befunde

3 Knochenneubildungen im Perilymph raum was auch bereits Wolff und Mitarbeiter ausgeführt haben

Der Endolymphhydrops ist demnach Folge der Grundkrankheit Dazu möchten wir fol gende morphologische Beiträge geben

1 Die perilymphatische Fibrose scheint die locale Endolymph–Perilymphpassage stark zu behindern Der Hydrops ist nach Jahnke mitbedingt durch eine Störung der Peri-lymph–Endolymphschranke

2 Die massiven Veränderungen um den Ductus reuniens werfen aber die immer wieder diskutierte Frage nach der longitudinalen Endolymphcirculation auf Eine Stenose Ver ziehung an dieser Engstelle kann Ursache sein für den starken Hydrops in der Cochlea so wie wir Verziehungen oder Blockade des häutigen Anfangsteiles des Ductus endolym-phaticus als Ursache für den Hydrops an den Strukturen des Vorhofs angegeben haben

3 Die perisacculäre Fibrose muß Einfluß haben auf die Resorption von Endolymphe

Acta Otolaryngol 89: 317–322, 1980

SENSITIVE PERIOD TO ACOUSTIC TRAUMA IN THE RAT PUP COCHLEA

Histological Findings

M. Lenoir and R. Pujol

From Laboratoire de Neurophysiologie Sensorielle, Université de Provence, Marseille, France

Abstract. In a previous paper we demonstrated physiologically a sensitive period for acoustic trauma in the rat pup cochlea, with a maximum at 22 days of age. Seven or 60 days after noise exposure these 22-day exposed rats were used for light and electron microscopy. At 7 days, surface preparation revealed restricted damage in the basal cochlear coil, while with electron microscopy signs of cytoplasmic degeneration appeared in the great majority of basal coil structures. Two months after noise exposure these structures have completely degenerated. These results were discussed in terms of mechanical versus metabolic explanations of the critical period for acoustic trauma.

A supra-normal period of sensitivity to acoustic trauma during development (discussed in a recent review by Saunders & Bock, 1978) has been physiologically demonstrated in kittens (Price, 1976) and hamsters (Bock & Saunders, 1977). It has also been histologically suggested in guinea pigs (Falk et al., 1974) and hypothesized in human babies (Douek et al., 1976). We investigated rats and found (Lenoir et al., 1979*a*) that, when exposed to 120 dB white noise lasting 30 minutes, rats 16 to 40 days of age showed strong permanent threshold shifts (PTS). PTSs were maximal for high frequency tones when animals were exposed at day 22. The finding of maximal sensitivity correlates well with data on rat cochlear maturation (Wada, 1923; Crowley & Hepp-Raymond, 1966; Lenoir et al., 1980*b*) which appears to end at about 20 days of age.

The present study complements our physiological findings (Lenoir et al., 1979). The purpose was to try to determine what kind of structures in the cochlea are responsible for the sensitive period to acoustic trauma. Light and electron microscopic data give a partial answer to this question.

METHODS

Six male white rats (Sprague Dawley) exposed at 22 days of age to a 120 dB SPL white noise and showing strong PTSs (60–80 dB between 6 and 20 kHz, for details see Lenoir et al., 1979) were investigated. Histological preparations were made either 7 days (3 rats) or 2 months (3 rats) after traumatic exposure. Animals were anaesthetized with an i.p. injection of Penthotal. The cochleae were quickly removed and then immersed in 1% osmium tetroxide solution for 1 h. After washing and dehydrating they were embedded in Spurr resin. Three types of histological techniques were used to study the cochleae: surface preparations (Spoendlin & Brun, 1973) giving an idea of cochleograms, semi-thin transverse sections, also checked by light microscopy, and ultra-thin sections mounted on Formvar coated grids and examined with a JEOL 100 C electron microscope.

RESULTS

1. *Seven days after traumatic exposure*

1.1. *Surface preparations.* Only the basal coil of the cochlea showed slight damage, other

This work was supported by grants from INSERM (CRL and ARS no. 6), DGRST (Actions BRD-DN) and CNRS (RCP no. 537).

Subotić to Zechner: Do you think that the connective tissue in the perilymphatic sphere is caused by the changes in labyrinthine circulation or by infection. I agree with you that the blockade of the endolymphatic duct in human temporal bones as in congenital malformations like *Mondini or in cases with temporal bone fracture causes a* hydrops which proves that the congenital flow does exist.

Zechner (Reply)

To Kelemen Thank you very much for your kind comments In Cogan s syndrome we had first-hand information from the Massachussets Eye and Ear Infirmary

To Hulting The reason for death in the pathology report was general arteriosclerosis within all areas of the body Cortisone treatment needs a high initial dosage but then we substitute partially with Endoxan[®] and at reasonable dosage we administer Imurex[®] instead of Endoxan[®]

To Subotić We saw no signs of labyrinthitis and the bone formation we calculate as ossification within the perilymphatic space after a special vascular disease The best sample of blockage is your paper on Mondini malformation



Figs 3–11 EM aspects of damaged structures in the basal coil of pup cochlea 7 days after traumatic exposure

Figs 3 4 Apical pole of OHCs. Note numerous lysosomes (*Ly*), vacuoles (*V*) and Golgi structures (*G*) 16 000

Figs 5 6 IHC showing the same cytoplasmic inclusions as in OHC. Note on Fig 5 damaged stereocilia (*arrow*) 10 000 (*5*), 17 000 (6).

Figs 7 8 Afferent fibres spiralling between Deiters' cells with vacuoles and myelin figures (*Mf*). 16 000 (*7*), 9 000 (*8*)

Figs 9 10 Efferent endings to OHC. Note the abnormally low density of synaptic vesicles and myelin figures (*Mf*). 15 000 (*9*), 16 000 (*10*)

Fig 11 Myelin figure complex (Spoendlin 1970) in supporting IHC 13 000

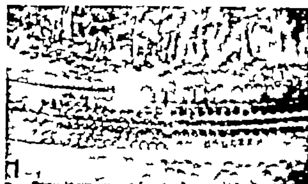


Fig 1 Surface preparation in the basal coil of rat pup cochlea 7 days after traumatic exposure Only very few small holes as the one marked by arrow were seen at OHC level Nomarski optics ×250.



Fig 2 Cross section made through the damaged area seen on fig 1 OHCs are missing and supporting cells hypertrophied Nomarski optics ×250.

coils looked normal with this technique In 3 or 4 areas small holes were noticed (Fig 1) each of which consisted of 1 to 8 missing outer hair cells (OHCs) Generally the corresponding external pillars were either missing or damaged Missing OHCs were most often seen in the first row

1 2 *Semi-thin sections* Transverse sections made on the localized damaged areas (see 1 1) confirmed the absence of OHCs and the damage to external pillars which appeared distorted or broken (Fig 2) Epithelial cells of the internal spiral sulcus and Hensen s cells were hypertrophied In neighbouring areas the cochlea looked normal in light microscopy except again for the hypertrophy of supporting cells

1 3 *Electron microscopic observations* Regardless of the place of transverse ultra-thin sectioning within the basal coil of the cochlea sensory and even neural structures presented signs of degeneration which of course were unnoticeable with light microscopy The great majority of OHCs were distorted and numerous cytoplasmic modifications appeared The apical pole of the cell just underneath the cuticular plate contained vacuoles lysosomes and dark mitochondria (Figs 3 4) Inner hair cells (IHCs) did not show any gross morphological changes but again cytoplasmic signs of metabolic impairments appeared similar to but less numerous than those seen in OHCs In addition IHCs showed an increase in endoplasmic reticulum forming Golgi-like apparatus and abnormal stereocilia (Figs 5 6)

Abnormalities were also found in neural endings inside the organ of Corti The majority of afferents below the OHCs spiralling between Deiter s cells showed vacuoles and myelin figures (Figs 7 8) Efferent endings to the OHCs also contained vacuoles and myelin figures The density of vesicles was abnormally low (Figs 9 10) At the level of IHCs the neural supply appeared normal

Cytoplasmic degenerating processes were also encountered in supporting cells (Fig. 11) The inner spiral sulcus cells showed besides the hypertrophy already mentioned cytoplasmic evaginations in the cochlear duct. In the external sulcus Hensen s cells and Claudius cells were also hypertrophied

2 *Two months after traumatic exposure*

2 1 *Surface preparation* Damage had now extended and about 80% of basal OHCs were missing the remaining 20% looked greatly disorganized No apparent lesions were seen at the IHC level

2 2 *Electron microscopic observations* Sensory and neural structures showed pronounced degeneration At locations where OHCs were present in surface preparations there remained only the cuticular plate with abnormal hairs and a small cytoplasmic portion IHCs now also appeared degenerated

the fact that Nuel's spaces which have just differentiated around day 15 place OHCs and their nerve endings directly in contact with a corticolymph possibly poisoned by endolymph In contrast, IHCs are much more protected throughout their life by neighbouring supporting cells.

Lastly vascular disorders (Lawrence 1970 Hawkins 1971) cannot be excluded when explaining the sensitive period to trauma. Again a strong correlation exists between that period and the time when cochlear vessels (spiral and strial ones) seem to switch from immature to mature stage (Wada 1923 Lenoir et al 1980) The characteristic decrease of vascularization may coincide with a peculiar weakness of sensory structures not yet metabolically stabilized

To account for the supra-normal sensitivity to acoustic trauma at 120 dB SPL these various hypotheses probably need to be considered together Experiments are now under way to try to determine the sensitive period more precisely by lowering and filtering the traumatic noise This is hopefully one way to discriminate among histological impairments which occur first and are responsible for immediate deafness and those which are secondary consequences and extend the apparent traumatized areas

ACKNOWLEDGEMENTS

W gratefully acknowledge A. Shnerson for assistance in rewriting the manuscript Thanks for technical assistance go t C Devigne and D Lombardo (histology), to L. Agats (photography) and to M. R. Ibrahim (typing).

RÉSUMÉ

Dans un article précédent, nous avons montre que la cochlée du raton présente un maximum de sensibilité au trauma acoustique au 22e jour postnatal. Sept et 60 jours après l'exposition au trauma des investigations histologiques ont été réalisées en microscopie optique et électronique. Sept jours après le trauma, les préparations de surface ne révèlent que des dégâts limités, alors qu'en microscopie électronique des signes de dégénérescence cytoplasmique apparaissent dans toutes les structures du tour basal de la cochlée. Ces résultats sont discutés en fonction des explications mécaniques ou métaboliques du trauma durant la période critique

ZUSAMMENFASSUNG

I einem vorhergehenden Artikel haben wir gezeigt daß die Cochlea der Jungratten am 22 Tage nach der Geburt eine Höchstempfindlichkeit gegen Hörschaden aufweist. Am 7 und 60 Tage nachdem die Jungratten des Traumas ausgesetzt wurden, wurden histologische Erforschungen mit dem optischen und elektronischen Mikroskop unternommen Am 7 Tage einen Präparate nur beschränkte Beschädigungen auf Hingegen zeigen Beobachtungen mit dem elektronischen Mikroskop Zeichen eines Verderbens des Zellplasmas in allen Strukturen in der Basis der Cochlea. Hier wollen wir diesen Befund in bezug auf die mechanischen und metabolischen Erscheinungen der Hörschäden in der kritischen eriode besprechen.

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Nerve fibres had completely disappeared at the OHC level with Deiter s cells filling the space left free by the degenerated sensory cells Below IHCs degeneration had occurred in the nerve supply and the retrograde process affected most of the laminar fibres

DISCUSSION

Apart from the mechanical lesions seen immediately after exposure to very intense sounds (around 130 dB SPL see Spoendlin & Brun 1973) the majority of traumatic processes in the cochlea originate from metabolic impairments According to Bohne (1976) three main factors may contribute to metabolic trauma enzymatic depletion within the sensory cells due to over functioning endolymph penetrating inside the organ of Corti via small holes in the reticular lamina and vascular disorders This kind of trauma is characterized by a slow and progressive degeneration of sensory and nerve structures which takes several months to fully develop (Bohne 1976 Spoendlin 1976) However electrophysiological and behavioral meausres show threshold elevations immediately following noise exposure (Hawkins et al 1976) The kinetics of the trauma found in our developmental experiment agree very well with these findings

The main observation in our previous physiological study (Lenoir et al 1979*a*) was of a period of heightened sensitivity to acoustic trauma at a time when the cochlea becomes just mature An important question arises can any of the above-mentioned explanations for acoustic trauma in the adult be applied to our findings of a critical period? The data reported here give a partial answer to this question

1 *Mechanical lesions*

During the critical period a half-hour of 120 dB SPL white noise seems enough to cause minor mechanical lesions in the organ of Corti Beside obvious damage represented by missing OHCs or external pillars there can be more discrete lesions on the reticular lamina which may be of major importance in the subsequent cochlear degeneration Actually endolymph penetration into the organ of Corti seems probable

2 *Metabolic impairments*

The first impression which arises from our electron microscopic and physiological data is of a kind of severe metabolic exhaustion of sensory cells in such a manner that recovery is impossible Cytoplasmic anomalies such as lysosomes myelin figures and vacuoles have been described in adult preparations as indicating an over functioning of hair cells (Engström & Ades 1960 Spoendlin 1970 Spoendlin & Brun 1973) It seems quite logical to think about an increase of this phenomenon in cochleae which have just reached their adult stage but may then not be completely stabilized in terms of their metabolic capacities There is also a possibility that the efferent endings which may be responsible for protecting the hair cells against an over-functioning are not mature enough in 22-day-old rats to play the same role as they do in adults We know that the large efferents to OHCs are among the last structures to develop in the cochlea (Kikuchi & Hilding 1965 Pujol et al 1978 Lenoir et al 1980) Some of the histological findings reported here inside efferent endings (myelin figures and the paucity of synaptic vesicles) suggest that the efferent system may have been activated too much for its developmental stage

Another aspect of metabolic impairment suggested by our results is a possible poisoning of the organ of Corti by endolymph This phenomenon originating from small mechanical lesions of the reticular lamina has been hypothesized in adult cochlea (Bohne 1974) To explain its increase during the sensitive period we have to suggest that the reticular lamina is not completely mature at that time However what leads us to a poisoning hypothesis is that outer structures are more damaged than inner ones This may be due to

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INVESTIGATIONS ON THE CRITICAL PERILYMPHATIC PRESSURE VALUE CAUSING ROUND WINDOW MEMBRANE RUPTURE IN ANESTHETIZED CATS

Ernő Miriszlai and Péter Sándor

From the ORL Department of the Second Pediatric Clinic and Experimental Research Department Semmelweis University Medical School, Budapest Hungary

Abstract Increasing perilymphatic fluid pressure was found to be an important factor in the etiology of round window membrane rupture. The critical pressure causing membrane rupture was determined in anesthetized cats. Its value was found to be in the range 10–30 mmHg ($\bar{x}\pm$S.E.M.=23.4±17.1) This value was compared with the pressure increases caused by thoracic and abdominal compression, cervical strangulation, forced Trendelenburg position, coughing and sneezing.

Although pressure and flow conditions in the perilymphatic space have been investigated from several aspects in the last couple of years one can find scarcely any reference in the literature about the critical perilymphatic pressure values causing rupture of the round window membrane

In the present series of experiments—carried out in anesthetized cats—we have investigated this critical range of the perilymphatic pressure

The pressure values causing rupture of the round window membrane were compared with those increased perilymphatic pressure values which could be measured in different, experimentally induced physiologic and pathophysiologic conditions in the same experimental animals.

METHODS

The experiments were performed on 12 female cats weighing 2½–3½ kg and anesthetized with 40 mg/kg sodium pentobarbital (Nembutal Abbott, Ottignies Belgium) intravenously.

The trachea was cannulated with a glass cannula, and the bullae osseae were prepared free on both sides from incisions led behind the ears The round window was exposed by opening the bulla. In one group of the animals (6 cats) a 1.5 mm hole was opened with a dental drill to the perilymphatic space through the bony wall of the round window at some millimetres distance from the window In another group of animals (6 cats) the perilymphatic space was opened by stapedectomy

A 1.0 mm outer diameter metal cannula was inserted into the hole and fixed with Duracryl (Duracryl "O" self-curing resin SPOFA-Dental Prague) dental cement. The cannula was connected to a short polyethylene tube, filled with Evans-blue stained physiological saline. The tube was connected to a three-way stopcock, the first branch of which was connected to a mercury manometer whose pressure values were recorded on a kymograph. The second branch was connected to an electric pressure transducer (P 23 AA Statham, Hato Rey Puerto Rico USA) in order to have a simultaneous possibility for recording low "break-through" pressure values on a polygraph (Officine Galileo R 105 h, O.T.E. Biomedica, Florence, Italy). The pressure in the perilymphatic space was raised slowly and continuously through the third branch of the stopcock with the aid of a Mantoux syringe filled with Evans-blue stained physiological saline

The experiments were carried out only in those animals in which the round window membrane was intact under the control of an operating microscope (×3[illegible] magnification)

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Marc Lenoir
Laboratoire de Neurophysiologie sensorielle
Université de Provence
Centre de St Jérôme
13397 Marseille
Cédex 4
F ance

DISCUSSION

Wersäll to Pujol You mentioned two morphological criteria of damage One was perforation of the organ of Corti One was changes in the amount of lysosomes Are your defects real holes or areas of degeneration of hair cells without holes Have you tried to quantify the damage inside the hair cell? Did you do any scanning EM

Spoendlin to Pujol The poisoning of the outer hair cells by endolymph which penetrates through holes and ruptures of the reticular lamina is certainly an interesting possibility However this could not explain the delayed degeneration of outer hair cells which you observe between one week and 2 months after the exposure Localized ruptures in the reticular lamina have a tendency to be closed by a scar very rapidly following the exposure

Verwoerd to Pujol What do you mean by "the last stages of cochlear maturation ? Did you attempt to repeatedly expose rats to noise during the critical period"

Pujol (Reply)

To Wersall and Spoendlin We intend to do some scanning EM which of course might give us a lot of information.

The structural impairments of hair cells and nerve endings shown in our EM 7 days after noise exposure indicate that a degenerating process is under way which will destroy the organ of Corti almost completely after months That process can be explained by the poisoning hypothesis But these impairments do not seem strong enough to account for the immediate deafness. Then, another explanation is that they can be signs of a severe metabolic exhaustion of structures just achieving their maturation and not completely yet stabilized

To Verwoerd The last stages of the maturation of the organ of Corti (which coincide with the beginning of the critical period) concern the OHC and their efferent innervation

I can answer indirectly as we haven t done a double exposure to noise But we have sensitized a cochlea (during the critical period) by low doses of antibiotics, the effect of noise exposure is then much more pronounced

The slowly developing process of the membrane rupture was recorded simultaneous with the pressure measurements by means of a Minolta XG_2 automatic camera combined with an auto-winder

Perilymphatic fluid pressure changes under the influence of cervical strangulation forced Trendelenburg position (Miriszlai 1977) chest and abdominal compression coughing and sneezing were tested in each experiment before measuring the critical rupture-causing pressure values

Cervical chest and abdominal compression was produced manually The forced Trendelenburg position was produced by lifting the hind limbs up to vertical position Coughing and sneezing was provoked by mechanical stimulation of the trachea and the nose with a polyethylene tube The maximum perilymphatic pressure changes measured during these model situations were compared with the critical pressure values causing round window membrane rupture

A detailed histologic (electronmicroscopic) study of the structure of the round window membrane was published by us previously (Miriszlai et al 1978) Since the situation and the extent of the membrane damages were quite different in each of the experiments histologic investigations were not carried out in connection with the present study

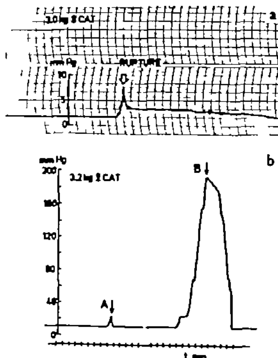


Fig 4 Original perilymphatic pressure recordings showing the moment of the round window membrane rupture in cat ( ) Electromanometric recording. (*b*) Recordings obtained in the two inner ears (*A* and *B*) of the same cat with mercury manometer ↓ The moment of the rupture

RESULTS

The dynamics of the round window membrane rupture are demonstrated in Figs. 1 2, and 3 respectively Fig 1 shows the appearance of the normal healthy round window membrane in the cat. Fig. 2 shows how the membrane swells out from the perilymphatic space under the influence of the increased pressure caused by the Evans blue stained saline injection. the membrane is reaching the stage prior to rupture Fig. 3 demonstrates the picture of the membrane rupture caused by an experimentally induced increase in the perilymphatic pressure

Fig. 4 demonstrates some typical original pressure recordings of the increasing perilymphatic fluid pressure and of the moment of membrane rupture Part (*a*) demonstrates an electromanometric recording in which the rupture of the membrane occurred at a pressure value which can be considered as an average rupture-causing pressure in the present experiments Part (*b*) shows two kymograph recordings (obtained with mercury manometer) from the two inner ears of the same animal. These recordings demonstrate the observation that the pressure value causing round window membrane rupture can be quite different in the two inner ears of the same animal

Perilymphatic pressure values causing the rupture of the round window membrane in the experiments are summarized in Table I As can be seen the critical average pressure value causing rupture of the round window mem-



Fig 1 Intact healthy round window membrane of the cat ×8.5



Fig 2 Round window membrane under the influence of critically increased perilymphatic fluid pressure ×4



Fig 3 Ruptured round window membrane of the cat. ×8.5

Table II *Maximum perilymphatic pressure increases (in mmHg) under the influence of test situations*

Type of the test	Cervical strangulation	Thoracic compression	Forced Trendelenburg position	Coughing	Sneezing
Number of exp animals	3 cats	7 cats	5 cats	5 cats	5 cats
Inner ears	8 ears	11 ears	10 ears	7 ears	5 ears
Measurements	9 cases	24 cases	22 cases	36 cases	29 cases
x	1.09	0 75	1 79	1 14	1 18
S E M	0.31	0.11	0.34	0.12	0 15
S D ±	0 93	0 53	1.58	0.72	0.80

sure changes are shown on Table II As can be seen in this table the most significant pressure increase was observed during the forced Trendelenburg position In some of these cases we have observed a pressure increase of 5.8 and 4 0 mmHg magnitude which values are close to the lowest pressure values causing round window membrane rupture in our experiments

DISCUSSION

The symptoms of the round window membrane rupture—according to the literature data—can be observed in the form of serious disturbances of the cochlear and vestibular function and ear noises (Simmons 1968 Goodhill 1971 1973 Pullen 1977 Freeman et al. 1974 Healy et al 1974 Taylor & Bricknell, 1976 Allam 1976 Althaus 1977 Goodman & Morioka, 1978 Kleinfeldt 1978 Molvaer et al. 1978 Behbehani & Kastenbauer 1978)

Experimental studies published in the last couple of years have provided a considerable amount of data on the pathophysiologic background of the disfunctions (Simmons et al. 1962 Arnold & Ilberg 1972, Lamkin et al., 1975 Axelsson et al 1977 Ivarsson & Pedersen, 1977 Weiskopf et al. 1978)

The data of Simmons (1962, 1968) and Goodhill (1971 1973) have called attention to the significance of the perilymphatic pressure changes in the development of perilymphatic fistulas The aim of our present investigations was to prove the connection between the perilymphatic pressure increase with the rupture of the round window membrane in experimental conditions and also to determine the pressure range of the perilymphatic fluid at which round window membrane rupture can occur The results of the animal experiments presented show that increased perilymphatic pressure can lead definitely to rupture of the round window membrane The average of the critical pressure values was between 20 and 30 mmHg. The highest and the lowest pressure values at which membrane rupture was observed in the experiments were 66 and 6 mmHg respectively In this pressure range there was no accompanying oval window membrane rupture This fact makes it certain that the tissue structure of the oval window membrane is more resistant to the increased pressure than is that of the round window membrane in spite of the observation that the tissue layer covering the perilymphatic space from inside appears to be a homogenous arachnoid structure (Franke 1979)

According to our present results, the pressure values causing rupture of the round window membrane can show considerable individual differences and significant differences were found in this respect even in the two inner ears of the same animal We assume that this phenomenon is probably due to the structural and mechanical resistance differences of the membranes. The perilymphatic pressure

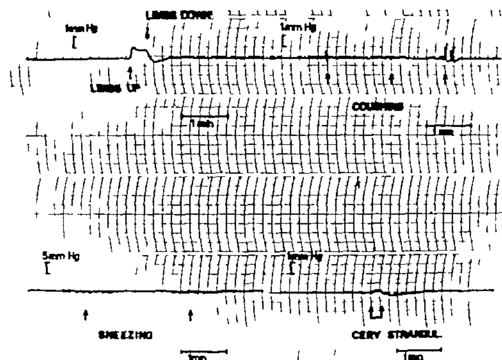


Fig 5 Original pressure re cordings, showing the in creased perilymphatic pres sure in the cat under the in fluence of (*a*) forced Tren delenburg position (hind limbs elevated to vertical position) (*b*) coughing, (*c*) sneezing, (*d*) cervical stran gulation

brane in 12 ears of 8 anesthetized cats is equal to 23 4±17 1 mmHg (x±S E M S D =4 9 mmHg) The upper and lower limits of the critical pressure values in healthy intact mem branes are 66 mmHg and 6 mmHg respective ly The rupture-causing critical pressure val ues can be quite different in the two ears of the same animal even in the case when both of the membranes were found healthy under the operating microscope

Table I *Critical perilymphatic pressure val ues causing round window membrane rupture in anesthetiˌed cats*

No of exp animal	Body weight (kg)	Pressure causing rupture (mmHg)
1	3.5	18 0
		66 0
	3 0	26 0
3	3 0	10.5
		22 0
4	3 0	L.5
		6 0
5	3.2	14 0
6	2.3	22.0
7	3.5	42 0
		34 0
8	.8	8.0

$\bar{x}$=23 4 mmHg S.E M =17 1 mmHg S D =4 9 mmHg n=12.

We were able to perform reliable pressure measurements on both inner ears of 4 animals. The average rupture-causing pressure was 17 1 mmHg on the one side and 35 6 mmHg on the other side of the same animal—thus rupture could be produced in these cats with a 18 5 mmHg pressure difference between the two ears The round window membrane was visibly enlarged in 4 inner ears of 4 animals (outside of the 8 healthy cats) in another series of our experiments

Membrane rupture was observed at 110 and at 186 mmHg in two of these membranes and in the other two ears it was not possible to produce a rupture even above 230 mmHg perilymphatic pressure In these latter two cases the microscopic observation of the en larged membranes showed an interesting pic ture the Evans blue stained saline penetrated between the layers of the round window mem brane and we could observe a suffusion of this fluid below the periosteum covering the bone around the window

An original electromanometric pressure re cording in Fig 5 demonstrates the pressure changes in the perilymphatic space under the influence of the pressure increasing test ma noeuvres The average values of these pres

DISCUSSION

Tonndorf to Miriszlai I wish you had divided your cases into tw groups, 1) in hich you raised the pressure ex tremely slowly so as to produce something like pure pressure effect, and) in hich you changed the pressure fast as you apparently did in your experiments, so as to obtain the effect of acceleration These are tw different things.

Sandor to Tonndorf You are right. However we found no differences between the critical pressure values when w have compared the effect of sudden pressure increase and pressure elevation caused by a very slow infusion pump Another aspect of this question. In the reality in those situations we tried to modelling here/coughing, sneezing/ the pressure increases even more rapidly as in the presented series of our experiments.

increases evoked by thoracic and abdominal compression coughing sneezing and forced Trendelenburg position caused no round window membrane rupture though the highest pressure values in these situations were close to the lowest rupture-causing perilymphatic pressure values *Hence the possibility that* these situations can lead to the rupture of the membrane after membrane injuries or in the case of disfunction of the tuba cannot be excluded These results provide data for an understanding of the clinical observations that physical effort situations can sometimes lead *to round window membrane rupture*

The results of our experiments are related to the fact that in the pressure increasing situations investigated by us the elevation of the pressure generally does not reach the upper limits of the tensile strength of the round window membrane The resistance of the intact healthy membrane structure against explosive everyday pressure increases (coughing sneezing) in the inner ear seems to be generally high

It seems very likely therefore that membrane rupture occurs only in those cases when an extreme pressure increase affects the damaged membrane of the round window

ACKNOWLEDGEMENT

This work was supported by NINCDS Grant (NS-10939-04) and by the Scientific Research Council Ministry of health Hungary (1-07-0301-00-1/K)

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E M ris lai M.D
Dept of Otolaryngology
II U iversity Children s Clinic
Bud pest
Tuzoltó 7 1094
Hungary



Abb. 1 (*a*) Zusammengesetzter Ausschnitt aus der Stria vascularis des Falles 1) Erste Windung: *P* Prominentia spiralis, *E*, flaches Restepithel; darunter unmittelbar angrenzend Ligamentum spirale (*L*). Im oberen Anteil besteht noch ein massives Striaödem (↗). Insgesamt aufgehobene Striamorphologie und Fehlen sämtlicher Kapillaren 460 (*b*) Zweite Windung: über der gesamten Striaausbreitung schweres Ödem mit Verlust aller charakteristischer Strukturen. 460

den Befunden bei der experimentellen Masugi-Nephritis den Einfluß von Nierenerkrankungen *auf das Innenohr zu erhellen.*

MATERIAL UND METHODIK

Die Felsenbeine zweier Patientinnen mit Alport Syndrom wurden in einem Falle 1,5 Stunden post mortem, im anderen Falle 45 Minuten post mortem transmeatal nach Herausnahme des Trommelfelles des Steigbügel und *nach Eröffnen des runden Fensters* durc Perfusion mit 3,5%igem eisgekühltem Glutaraldehyd perfundiert Nach der Sektio

Für hervorragende technische Mitarbeit danke ich F
S. Linnenkohl.

Acta Otolaryngol 89 330–341 1980

ÜBERLEGUNGEN ZUR PATHOGENESE DES COCHLEO-RENALEN SYNDROMS[1]

Considerations on the Pathogenesis of the Cochleo-renal S\ndrome

W Arnold

Aus de Hals-Nasen-Ohrenklinik der Universität Düsseldorf BRD

Es ist bekannt daß bestimmte Nierenerkrankungen mit einer zunehmenden Innenohrschwerhörigkeit einhergehen Das Paradebeispiel hierfür stellt das Alport Syndrom dar eine familiäre Glomerulonephritis mit Innenohrschwerhörigkeit und Augenveränderungen Es ist dabei noch nicht bewiesen ob die genetische Determination von vorneherein eine Degeneration bestimmter Innenohranteile mit einbezieht oder ob die Innenohrschwerhörigkeit Folge einer besonderen Form von Glomerulonephritis ist Bei anderen Innenohrschwerhörigkeiten im Gefolge einer Glomerulonephritis wie beispielsweise bei der diabetischen Glomerulonephritis (Kimmelstiel-Wison) mag die Innenohrschwerhörigkeit sich aus der für die diabetische Grundkrankheit bekannten Basalmembranveränderung erklären Ferner führen besonders Medikamente die zur Therapie von Nierenfunktionsstörungen eingesetzt werden (Furosemide Ethacrynsäure) zu vorerst reversiblen bei langzeitiger Anwendung irreversiblen Hörstörungen Die Vorstellung von einer immunologisch bedingten Schwerhörigkeit (Quick 1975) begründet sich dagegen in der Beobachtung daß nierentransplantierte Patienten gelegentlich einen Innenohrhörverlust aufweisen Oda et al (1974) untersuchten 290 Patienten mit Nierentransplantationen beziehungsweise Patienten die unter Hämodialyse standen und fanden bei 43 Patienten einen eindeutigen Hörverlust in unmittelbarer Abhängigkeit von der Therapie der Nierenerkrankung Bei der Untersuchung von 16 Felsenbeinen von 8 Hämodialyse oder Nierentransplantations Patienten fanden sie unter den Felsenbeinen Ablagerungen im Bereich der Stria vascularis und degenerative Veränderungen der vestibulären Rezeptoren Ähnliche Ablagerungen hatte Gacek (1971) bereits beim Alport Syndrom beschrieben Da diese Ablagerungen auch bei anderen angeborenen oder genetisch bedingten Schwerhörigkeiten gesehen werden (Jervell-Lange Nielsen Syndrom Scheibe-Dysplasie) kann daraus offensichtlich keine spezifische Veränderung abgeleitet werden Vielmehr muß man bei der Betrachtung einer gemeinsamen oder voneinander abhängigen Reaktion von Nierengewebe und Innenohrgewebe entweder davon ausgehen daß es sowohl im glomerulären Nierensystem wie auch am Innenohr (hier vorzugsweise im Bereich der Stria vascularis) bestimmte Rezeptoren beispielsweise für Pharmaka gibt die nur hier oder hier besonders stark angreifen Oder aber es muß ausschließlich im Bereich der Nierenglomerula und der Stria vascularis immunologisch voneinander abhängige Gewebskomponenten geben die gemeinsam reagieren (Quick et al 1973 Arnold et al. 1976 Werdauer et al 1977)

Die licht und elektronenmikroskopische Untersuchung zweier Fälle von Alport Syndrom bei denen die Diagnose sowohl familienanamnestisch durch Nierenbiopsien bei verschiedenen Familienangehörigen sowie durch otologische Untersuchungen gestellt worden war kann dazu beitragen im Vergleich mit

[1] Mit Unterstützung der Deutschen Forschungsgemeinschaft (Ar 170/1)

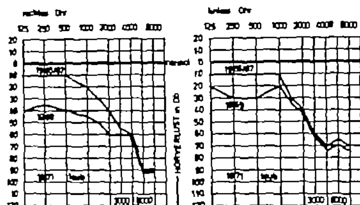


Abb 4 Tonschw Benaudiogramme 1966 1969 und 1971 I den letzten Lebensmonaten war die Patientin taub

der Stria vascularis aber nur eine geringfügige Degeneration der Prominentia spiralis Lediglich im Bereich der dritten Windung fanden sich noch Bezirke von epithelähnlichem Aufbau mit weitgestellten Gefäßen. Der Flüssigkeitsraum des nur mehr spärlich vorhandenen Ligamentum spirale erstreckte sich bis unmittelbar unter die Epithelreste (Abb 1*a b*) Die Reissner sche Membran war intakt, im Bereich des Limbus spiralis imponierte ein Kondensat von PAS-positivem Material an der Oberfläche der Interdentalzellen Auch die Tectorialmembran färbte sich intensiv PAS-positiv in ihren Randbezirken an. Das Corti sche Organ wies in allen Windungsanteilen einen vollständigen Verlust der inneren und äußeren Haarzellen auf, die Tunnelradialfasern fehlten in den Serienschnitten (Abb 2) Das Ganglion spirale ließ eine Degeneration der Nervenzellen erkennen wobei die umhüllenden Mantelzellen erhalten blieben und somit häufig lochförmige Areale zu erkennen waren (Abb 3) Tonaudiometrisch bestand bei dieser 38-jährigen Patientin zum Todeszeitpunkt eine beidseitige Taubheit (Abb 4)

Fall N 2

Bei dieser 42 jährigen Patientin mit familiärem Alport Syndrom war mehrere Jahre vor dem Tod durch eine Nierenbiopsie durch die klinische Symptomatik einschließlich eines geringfügig zunehmenden Hochtonverlustes und einer Linsentrübung die Diagnose bestätigt worden Die Patientin verstarb im Zustand der kompensierten Niereninsuffizienz an einem akuten Herzinfarkt Sie erhielt während der gesamten Erkrankungsjahre keine innenohrtoxischespharmaka. Die Nierenbiopsie 1974 ließ im Bereich der Nierenglomerula noch eine Verbreiterung der Basalmembranen sowie eine Verschmelzung der epithelialen Zellausläufer erkennen die nach David et al (1966) und nach Zollinger & Mihatsch (1978) kennzeichnend für glomeruläre Veränderungen beim Alport Syndrom sind (Abb 5*a b*) Zum Zeitpunkt dieser Nierenbiopsie wies die Patientin eine äußerst geringgradige vorwiegend im Hochtonbereich gelagerte Innenohrschwerhörigkeit mit deutlichem *Seitenunterschied* auf In den folgenden Jahren verschlechterte sich die Nierensituation und *gleichzeitig* kam es von einer geringgradigen Schwerhörigkeit *zu einer mittelgradigen* wiederum seitenunterschiedlichen Schwerhörigkeit dabei war die stärkste Zunahme des Hörverlustes im Hochtonbereich vorhanden (Abb 6)

Die Untersuchung der Nierenglomerula zum Todeszeitpunkt ließ eine weitgehende Degeneration und Obliteration erkennen elektronenmikroskopisch waren die meisten Gefäße durch eine enorme Proliferation der Epithelien und Verdickung der Basalmembranen eingeengt oder obliteriert (Abb 7)

Die Stria vascularis war in allen Windungsanteilen voll erhalten ausschließlich im Bereich der Reissner schen Membran und unmittelbar



Abb 2 Cortiorgan der zweiten Windung Degeneration der Inneren und Äußeren Haarzellen Verlust der Sinneshaare und der meisten Stützelemente ×423

wurden die Felsenbeine entnommen so zugesägt daß die Schnecken in einem 1,5 ccm großen Würfel enthalten waren und erneut über Nacht in 3 5%igem Glutaraldehyd nach fixiert Anschließend wurden die Cochleae mit 1%igem Osmiumtetroxyd nachfixiert Im ersten Fall wurde anschließend mit EDTA entkalkt die Schnecke in parallele Scheiben zerschnitten und eingebettet im zweiten Falle wurde ohne Entkalkung in hartes Epon eingebettet und der Eponblock anschließend mit einer hochtourigen Säge in millimeterdicke Scheiben zerschnitten Die Semidünnschnitte wurden mit Methylenblau oder nach Mallory II gefärbt Die Kontrastierung der elektronenmikroskopischen Schnitte erfolgte mit Uranyl acetat und Bleicitrat Die Untersuchung erfolgte an einem Elektronenmikroskop Zeiss EM 9 a.

Fall Nr 1 wurde bereits 1976 publiziert (H Weidauer W Arnold *Z Laryngol Rhinol* 55 6–16 1976)

BEFUNDE

Fall Nr 1

Histologisch bestand hier eine diffuse Glomerulonephritis mit enormer Verdickung der Basalmembranen so daß im PAS gefärbten Präparat die Gefäßlumina weitgehend verdrängt waren In großen Arealen waren die Glomerula vollständig obliteriert

Das Innenohr zeigte im Bereich aller Windungsanteile eine vollständige Degeneration

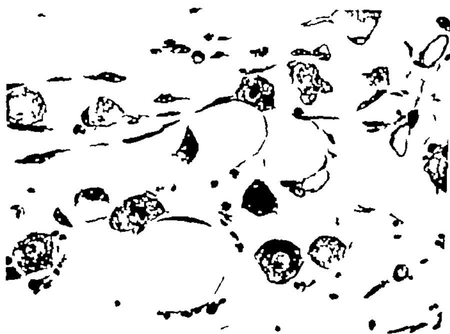


Abb 3 Ausschnitt aus dem Spiralganglion der zweiten Windung Anzahl der Ganglienzellen deutlich reduziert Zellen selbst pyknotisch oder verschwunden so daß leere, von Mantelzellen umgebene Hohlräume resultieren Den Ganglienzellen fehlt jegliche Myelinisation was nach Kimura für das menschliche Spiralganglion charakteristisch ist ×350

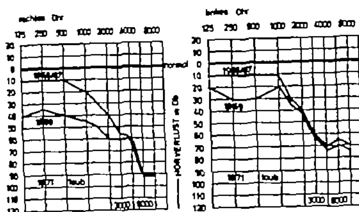


Abb. 4. Tonschwellenaudiogramme 1966, 1969 und 1971. In den letzten Lebensmonaten war die Patientin taub

der Stria vascularis, aber nur eine geringfügige Degeneration der Prominentia spiralis. Lediglich im Bereich der dritten Windung fanden sich noch Bezirke von epithelähnlichem Aufbau mit weitgestellten Gefäßen. Der Flüssigkeitsraum des nur mehr spärlich vorhandenen Ligamentum spirale erstreckte sich bis unmittelbar unter die Epithelreste (Abb. 1*a, b*). Die Reissner'sche Membran war intakt. Im Bereich des Limbus spiralis imponierte ein Kondensat von PAS-positivem Material an der Oberfläche der Interdentalzellen. Auch die Tectorialmembran färbte sich intensiv PAS-positiv in ihren Randbezirken an. Das Corti'sche Organ wies in allen Windungsanteilen einen vollständigen Verlust der inneren und äußeren Haarzellen auf; die Tunnelradialfasern fehlten in den Serienschnitten (Abb. 2). Das Ganglion spirale ließ eine Degeneration der Nervenzellen erkennen, wobei die umhüllenden Mantelzellen erhalten blieben und somit häufig lochförmige Areale zu erkennen waren (Abb. 3). Tonaudiometrisch bestand bei dieser 38-jährigen Patientin zum Todeszeitpunkt eine beidseitige Taubheit (Abb. 4).

Fall Nr. 2

Bei dieser 42-jährigen Patientin mit familiärem Alport Syndrom war mehrere Jahre vor dem Tod durch eine Nierenbiopsie, durch die klinische Symptomatik einschließlich eines geringfügig zunehmenden Hochtonverlustes und einer Linsentrübung die Diagnose bestätigt worden. Die Patientin verstarb im Zustand der kompensierten Niereninsuffizienz an einem akuten Herzinfarkt. Sie erhielt während der gesamten Erkrankungsjahre keine innenohrtoxischen Pharmaka. Die Nierenbiopsie 1974 ließ im Bereich der Nierenglomerula noch eine Verbreiterung der Basalmembranen sowie eine Verschmelzung der epithelialen Zellausläufer erkennen, die nach David et al. (1966) und nach Zollinger & Mihatsch (1978) kennzeichnend für glomeruläre Veränderungen beim Alport Syndrom sind (Abb. 5*a, b*). Zum Zeitpunkt dieser Nierenbiopsie wies die Patientin eine äußerst geringgradige, vorwiegend im Hochtonbereich gelagerte Innenohrschwerhörigkeit mit deutlichem Seitenunterschied auf. In den folgenden Jahren verschlechterte sich die Nierensituation und gleichzeitig kam es von einer geringgradigen Schwerhörigkeit zu einer mittelgradigen, wiederum seitenunterschiedlichen Schwerhörigkeit, dabei war die stärkste Zunahme des Hörverlustes im Hochtonbereich vorhanden (Abb. 6).

Die Untersuchung der Nierenglomerula zum Todeszeitpunkt ließ eine weitgehende Degeneration und Obliteration erkennen; elektronenmikroskopisch waren die meisten Gefäße durch eine enorme Proliferation der Epithelien und Verdickung der Basalmembranen eingeengt oder obliteriert (Abb. 7).

Die Stria vascularis war in allen Windungsanteilen voll erhalten, ausschließlich im Bereich der Reissner'schen Membran und unmittelbar



Abb 5(*a*) Glomerulumausschnitt mit zwei Schlingenlumina (*L*) die allseits von fusionierten teilweise breitflächig aufsitzenden Podocytenfortsätzen abgedeckt sind (↗) Nierenbiopsie 2 Jahre vor dem Tode ×4500 (*b*) Ausschnitt der Glomerulakapillarwand mit beginnender Verschmelzung plumper Podocytenzellfortsätze (*P*) ×5000

K J. 4 7 36 9.10 78 Alport Syndrom Drskr 100%

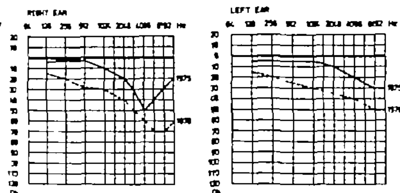


Abb 6 Tonschwellenaudiogramm 1975 1978 Die Patientin hatte bis zum Tode ein gutes Sprachverständnis

nahe der Prominentia spiralis bestand ein breites Interzellularoedem An der Oberfläche finden sich Protoplasmaprotrusionen und schaumige Zellauftreibungen die als pathologische Veränderungen betrachtet werden müssen (Abb 8*a* *b* *c*) In Serienschnitten waren die äußeren Haarzellen bis auf wenige Abschnitte im Bereich der Basalwindung in allen



Abb 7 Nierenbiopsie zum Todeszeitpunkt. Weitgehende Degeneration und Obliteration der Glomerulumschlingen, fehlende Gefäßlumina, Ersatz des freien Raumes durch Basalmembransubstanz (*B*). *K* Kapillarreste *BK* Bowman sche Kapsel 4 806.

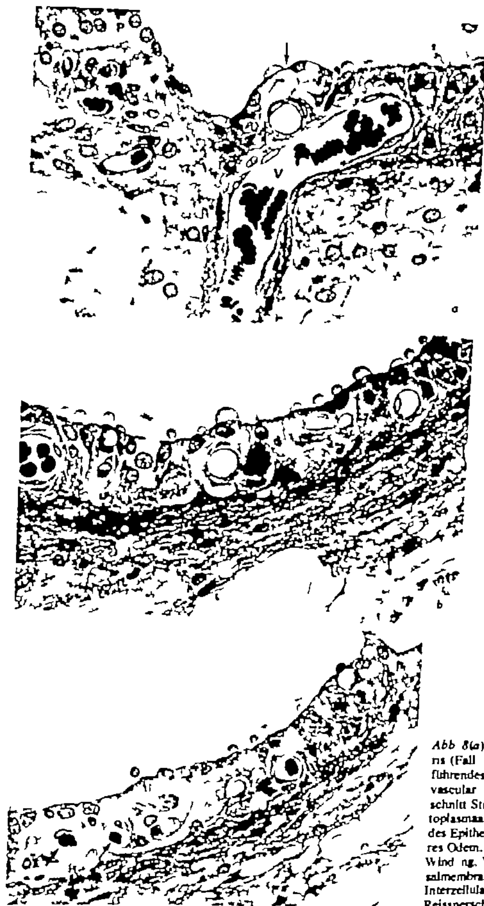


Abb 8(*a*) Ausschnitt aus der Stria vascularis (Fall) *P* Prominentia spiralis V abführendes venöses Gefäß (↗) Ödem perivascular Basalwindung ×580 (*b*) Ausschnitt Stria vascularis Basalwindung. Protoplasmaabschnürungen an der Oberfläche des Epithels vor allem basales perivasculäres Ödem. ×465 (*c*) Stria vascularis zweite Windung. Verbreiterung der kapillären Basalmembranen (↗) deutlich abgegrenztes Interzellularödem (+) nahe dem Ansatz der Reissnerschen Membran (*RM*) ×415

Abb. 9(*a*) Corti-Organ der zweiten Windung. Degenerative Veränderungen der Inneren Haarzelle (*IHZ*) bei normaler Struktur der Äußeren Haarzellen (*ÄHZ*) ×485 (*b*) Corti-Organ der Basalwindung. Äußere Haarzellen intakt, Innere Haarzelle pyknotisch (↗) Sinneshaare einer *Äußeren Haarzelle haften an Tecto*rialmembran. ×485



Abb. 10 Ausschnitt aus dem Ganglion *spirale der Basalwindung*. Normale Struktur; fehlende Myelinisation ist nicht pathologisch. [illegible]



Abb 11 Elektronenoptischer Aussch aus der Stria vascularis der Basalwindt Deutlicher als im Lichtmikroskop wird l das ausgedehnte Interzellularödem geschlossener epithelialer Oberfläc Auch die Basalzellschicht ist aufgelock (*BZ*) ×4 510

weiteren Windungsanteilen voll erhalten in der Basalwindung fehlten die inneren Haarzellen in den höheren Windungsanteilen waren die inneren Haarzellen vacuolig aufgetrieben und im Zustand der Degeneration Die Tunnelradialfasern waren voll erhalten die *Spiralganglienzellen* zeigten sowohl bezüglich der charakteristischen Morphologie als auch bezüglich der Gesamtpopulation keine auffallenden Veränderungen (Abb 9*a b* 10)

Elektronenmikroskopisch wurde im Bereich der Stria vascularis das interzelluläre Oedem deutlicher als im Lichtmikroskop auffallend war jedoch eine starke Verdickung der Basalmembranen der Striagefäße (Abb 11 12) Das letzte Tonschwellenaudiogramm drei Monate vor dem Tode zeigte eine gering bis mittelgradige fast pancochleäre Schwerhörigkeit die Diskrimination betrug 100% das Recruitment war positiv Von seiten des Vestibularorgans wurden keine Beschwerden beklag ein Spontan oder Provokationsnystagmus b stand nicht

DISKUSSION

Vergleicht man diese Befunde beim Alport Syndrom mit den Ergebnissen der experimentellen Masugi Nephritis (Quick 1975 Arnol et al 1976) so haben beide folgendes gemeinsam Antikörper gegen Basalmembranen bei der experimentellen Masugi Nephritis bewirken eine Anschwellung der Basalmembranen der Nierenglomerula und ein Zusammenfließen der epithelialen Ausläufer der Nierenglomerula Ferner kommt es im Innenohr ausschließlich zu einer immensen Verdickung der Basalmembranen im Bereich der Striakapillaren Veränderungen am Corti schen Organ oder an den Gaglienzellen des Ganglion spirale wurden im Kurzzeitversuch nicht gesehen



Abb 12 Lamellierung und massive Verbreiterung der Basalmembran einer Striakapillare perivaskuläres Ödem, an einer Stelle zieht die Basalmembran zwischen die Ausläufer der Marginalzellen (↗). 25 000

Beim Alport Syndrom kommt es ebenfalls zu Beginn der klinischen Manifestation zu einer Verbreiterung der glomerulären Basalmembranen mit Verschmelzung der epithelialen Füßchen Im Spätstadium des nephrotischen Syndroms sind die Basalmembranen extrem verdichtet und aufgetrieben daneben findet man eine Proliferation der Epithelzellen Im Bereich der Stria vascularis fällt ein interzelluläres Oedem nahe der Prominentia spiralis und nahe dem Ansatz der Reissner'schen Membran auf daneben eine deutliche Verdichtung und Verdickung der Basalmembranen um die Strgefäße. Schließlich beobachtet man degenerative Veränderungen der Epithelzellen die bei schwerem Verlauf der Erkrankung zu einer kompletten Degeneration der Stria vascularis führen Diese Veränderungen erscheinen zunächst die stärkste Auswirkung auf die innere Haarzelle zu haben, da diese vorwiegend in den basalen Windungsanteilen deutliche morphologische Schäden aufweisen. Die Spiralganglienzellen dagegen waren in dem vorliegenden Fall intakt Erst im Spätstadium der Erkrankung wenn die komplette Striadegeneration eingetreten ist wird auch ein allgemeiner Haarzellverlust und eine womöglich retrograde Degeneration der Spiralganglienzellen erkenntlich Entsprechend findet man im Tonschwellenaudiogramm zu Anfang der Erkrankung einen Hochtonverlust der Schallempfindungsverlust im *Mittelfrequenzbereich* mag bei 100%igem Diskriminationsvermögen als Folge der frühzeitigen strialen Schädigung aufzufassen sein

Da nach Spichtin & Mihatsch (1979) die Erkrankung meist in früher Kindheit mit Mikro- oder Makrohämaturie sehr oft im Anschluß an einen respiratorischen Infekt (33%) der oberen Luftwege beginnt und die Veränderungen am Innenohr offensichtlich eine Spätfolge der Erkrankung darstellen ist zu überlegen ob die Innenohrveränderungen beim Alport Syndrom nicht Folgen der primären Nierenschädigung sind und weniger als *Ausdruck* einer genetischen Determination aufzufassen sind Ob dabei diese Schädigung wie beispielsweise bei der experimentellen Masugi-Nephritis, Folge einer durch exogene Beeinflussung induzierten immunologischen Reak

tionsweise ist (gemeinsame Antigenizität von Nierenglomerulabasalmembranen und Basalmembranen der Striagefäße) oder aber die Nierenaffektion am Beginn der Erkrankung steht und erst im Laufe der Zeit auf immunologischem oder direkt toxischem Wege (vergl Oda et al 1974) das Innenohr beeinflußt wird kann vorerst nicht entschieden werden Es spricht manches dafür daß beim Alport Syndrom immunologische Reaktionsmechanismen ablaufen Es wäre auch vorstellbar daß bei dieser Erkrankung ein recht ähnlicher Pathomechanismus vorhanden ist wie er derzeit für den Rheumatismus (vergl Laine 1979) oder für bestimmte Formen des Diabetes erörtert wird Gerade die Tatsache daß Infekte die primäre Nierensymptomatik auslösen (Zollinger & Mihatsch 1978) deuten auf eine exogen induzierte Autoimmunkrankheit hin die – da familiär gehäuft auftretend – einer gewissen Prädisposition bedarf (ähnlich Diabetes Rheumatismus)

Überblickt man die anstehende Literatur über die wenigen sporadischen Beobachtungen bei Fällen von Alport Syndrom (Übersicht bei Schuknecht 1974) so vermißt man regelmäßig zu beobachtende pathologische Veränderungen am Innenohr wogegen die Veränderungen an der Niere als charakteristisch eingeordnet werden können Unter den Innenohrveränderungen findet man Ganglienzellverlust Atrophie des Ligamentum spirale Haarzellverlust Striaatrophie oder basophile Ablagerungen in der substrialen Zone Gerade letztere sind auch bei verschiedenen anderen Innenohrerkrankungen beobachtet worden (vergl LaVonne Bergstrom et al 1979)

Es läßt sich abschließend feststellen daß die schwersten Veränderungen am Innenohr bei nierenbeidingtem Grundleiden regelmäßig an der Stria vascularis zu suchen sind Man darf daraus folgern daß bestimmte Nierenerkrankungen zuerst zu einer Schädigung der Stria vascularis führen wobei der Pathomechanismus sowohl auf direkt toxischem wie auch auf immunologischem Wege ablaufen kann Die weiteren Veränderungen am Innenohr wie Untergang von Haarzellen oder Verlust cochleärer Neurone sind entweder sekundäre Folgen der Striadegeneration oder unmittelbare Folgen nierenbedingter Störungen des Innenohrmetabolismus Darauf deuten vor allem die Beobachtungen von Schreiner oder McCabe (persönliche Mitteilungen) hin die beim Alport Syndrom und anderen Nierenerkrankungen die mit Innenohrschwerhörigkeit einhergehen unter immunsuppressiver Therapie (z B Cortison) einen Wiederanstieg der Hörfunktion beobachteten Dies ist nur möglich, wenn zum Zeitpunkt des Therapiebeginnes die sensorischen und neuronalen Komponenten des Innenohres noch intakt waren da neuronale Elemente im Gegensatz zur Stria vascularis nicht mehr regenerationsfähig sind Diese Beobachtungen sprechen auch gegen eine genetisch determinierte Innenohrdegeneration beim Alport Syndrom

RÉSUMÉ

Les lésions histologiques du rein et de l'oreille interne sont démonstrées dans deux cas de syndrôme d'Alport. Les alterations de l'oreille interne sont répresentées au voisinage des capillaires striales et dans les cellules d'organ de Corti. Les lésions de la cochlea sont – après notre impression – la conséquence de la glomérulonéphrite

SUMMARY

The histological findings in two cases of *Alport s* syndrome are presented Damage within the inner ear is mainly represented at the vascular stria region and it seems that *the inner ear damage pattern* tallies with the renal alterations Glomerular basement thickening and splitting are the first and pathognomonic findings together with confluence of the epithelial feet. Later on, the increase in volume in the epithelial cells and the tremendous enlargement of the glomerular basement membranes leads to an obliteration of glomerular capillaries. In the first described case a subtotal *loss* of stria vascularis seems to be the reason for a degeneration of the inner and outer hair cells as well as of some spiral ganglion *cells The second case* revealed only a slight edema of the stria vascularis and apical protrusions of cytoplasm sometimes vacuolated Whereas in the basal turn most of the inner hair cells had disappeared in the upper turns we observed a slight degeneration of the inner hair cell but intact outer hair cell ystem Although in Alport syndrome there are—according to the literature—no constant findings, *our observations* allow us to assume that inner ear damage in the cochleo-renal yndrome is secondary to the kidney disease

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W Arnold M.D
Hals-Nasen-Ohrenklinik
der Universität Düsseldorf
D-4000 Düsseldorf
Moorenstraße 5
Deutschland

DISCUSSION

Kelemen to Arnold Emphasizes scepticism regarding the dilatation theory in Meniere and other conditions.

Temporal bone histology requires an equally careful preparation whether for light or electron microscopy

tionsweise ist (gemeinsame Antigenizität von Nierenglomerulabasalmembranen und Basal membranen der Striagefäße) oder aber die Nierenaffektion am Beginn der Erkrankung steht und erst im Laufe der Zeit auf immunologischem oder direkt toxischem Wege (vergl Oda et al 1974) das Innenohr beeinflußt wird kann vorerst nicht entschieden werden Es spricht manches dafür daß beim Alport Syndrom immunologische Reaktionsmechanismen ablaufen Es wäre auch vorstellbar daß bei dieser Erkrankung ein recht ähnlicher Pathomechanismus vorhanden ist wie er derzeit für den Rheumatismus (vergl Laine 1979) oder für bestimmte Formen des Diabetes erörtert wird Gerade die Tatsache daß Infekte die primäre Nierensymptomatik auslösen (Zollinger & Mihatsch 1978) deuten auf eine exogen induzierte Autoimmunkrankheit hin die – da familiär gehäuft auftretend – einer gewissen Prädisposition bedarf (ähnlich Diabetes Rheumatismus)

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Acta Otolaryngol 89 345–357 1980

PURETONE THRESHOLD ESTIMATION FROM ACOUSTIC REFLEX THRESHOLDS—A MYTH?

M L. Hyde P W Alberti P P Morgan F Symons and F Cummings

From the Silverman Hearing Research Laboratory Department of Otolaryngology Mount Sinai Hospital University of Toronto Toronto Canada

Abstract There have been repeated claims in the literature that hearing thresholds can be estimated by means of acoustic reflex threshold (ART) measurements W ha evaluated this thesis in depth by xamining the statistical relationships between conventional puretone thresholds and ART for tonal and broadband stimuli in large sample (1 707) of adult patients with sensorineural hearing loss, they were compensation claimants for noise induced loss. All relevant data were stored in computer database and analysed using the Statistical Package for the Social Sciences (SPSS). The relationships were examined from tw distinct standpoints, dependency of ART upon puretone thresholds and prediction of audiogram features using ART The average tonal ART is constant up to 70 dB average loss, and thereafter increases rapidly The broadband noise ART increases steadily for average losses of 10 to 85 dB The convergence principle therefore valid up to 70 dB in this population. The predictor suggested by Niemeyer & Sesterhenn does not fit the data The single ART hich best predicts verage loss is that for hite noise but multiple regression prediction using the hite noise and 500 Hz ART is preferable Predicti accuracy is dependent upon audiogram slope but the latter cannot be predicted usefully from the ART measures used. None of the predictors studied is adequate for medico-legal assessment and the method is probably not sufficiently accurate for clinical use in adults

Prediction of auditory puretone thresholds (PTT) from acoustic reflex threshold (ART) measurements has intrigued auditory scientists since it became practical to measure the acoustic reflex Potentially it provides an objective technique which is cheaper quicker and simpler to perform than various methods of electric response audiometry (ERA)

Niemeyer & Sesterhenn (1974) first proposed an empirical prediction equation for the PTT which depends upon a systematic decrease in the difference between wideband and puretone ARTs with increasing hearing loss This method has been expanded by various authors (Jerger et al 1974 Handler & Margolis 1977 Keith 1977 Van Wagoner & Goodwine 1977 Miller et al 1976)

The present authors take issue with many of the published reports.

Firstly there is relatively little use of statistical methods Prediction for example by multiple repression analysis or by discriminant analysis is a powerful and well developed area of statistical theory and practice These methods provide a meaningful way of quantifying predictor performance and force explicit mathematical modelling of the phenomena. Empirical approaches on the other hand are prone to subjective bias and irreproducibility

Secondly there seems to be a general lack of rigor concerning errors in prediction If a prediction of a numerical loss value is made then the method can be evaluated in terms of bias and variability of predictive errors

Thirdly we disagree with the concept of categorization of hearing loss data, used by several authors on the grounds that it is simplistic The intent of a prediction method is to obtain some estimate of hearing loss the latter is of the highest possible measurement strength namely a ratio scale variable Average hearing loss for example is not naturally categorical and to impose a categorization is to degrade it, with loss of information Statistical methods for deriving eval

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Supported in part by generous grant from the Workmens Compensation Board of Ontario.

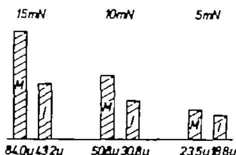


Fig 3 Average value

Static examinations into the stability of the malleus–incus joint were also highly conclusive It is a well-established and generally recognized fact that this joint is not flexible and of no importance for the transmission of oscillations It was possible to clearly check this stability and/or flexibility If for example a force is applied at two points with the same lever arm (Fig 1 upper left) the displacement must be the same—if the joint is inelastic However tests in 10 temporal bone preparations showed differences at forces of 5 10 and 15 mN The malleus could be moved more than the incus and the differences were the more pronounced the greater the forces applied were (Fig 3) From this we can see that this joint is by no means inelastic when such forces are applied If we reduce the force to 2 mN we still can measure a displacement difference of 1 to 1 2 only at 1 mN were the displacements of malleus and incus equal From this we can conclude that if forces of below 1 mN act there is no transmission loss which however can be certainly assumed to exist if the force is greater

Static measurements cannot be compared with dynamic ones without reservation but we can compare the forces which are active Thus 1 mN corresponds to 100 dyn which—related to the tympanum of about 50 mm²—correspond to a volume of 170 dB This figure tells us that within the human auditory area there is no loss of oscillation energy in the malleus–incus joint However in the case of great sound intensities i e above the pain threshold the joint becomes more and more elastic whereby the oscillation of the incus does not increase in the same extent as that of the malleus We may interpret this as being a function of sound protection

W Cancura
Univer HNO
Alserstr 4
A 1090 Wien
Austria

DISCUSSION

Tonndorf to Cancura There is a great need for static measurements for the construction of middle-ear prostheses.

There is no dispute between Békésy and us with respect to the mode of mallear displacement only to that of the tympanic membrane

I would urge you to repeat your measurements in living cats and then follow up after sacrifice Impedance changes rapidly after death and so should the material constant.

There is movement in the incudomallear joint at $f > 100$ H_z, but it is amplitude-independent.

Cancura (Reply):

Influences caused by ageing or drying were naturally examined No significant differences were ascertained as long as the preparations were kept under optimal conditions and it was easily possible to reproduce all measurements Variations occurred only when the ligaments started to disintegrate e g. through putrefaction.

Table I *Summary statistics for puretone AC thresholds*

Frequency (kHz)	Right ear *N*	Mean	Median	S.D	Left ear *N*	Mean	Median	S.D
0.5	1 203	25.2	22.7	13.[illegible]	1 203	25.8	23.5	13.4
1.0	1 206	36.3	36.3	15.2	1 204	36.9	37.7	15.8
2.0	1 204	52.1	53.6	14.3	1 203	54.1	55.4	13.9
3.0	1 203	[illegible]	63.4	13.8	1 198	64.2	64.4	13.[illegible]
4.0	1 195	68.3	68.1	14.4	1 195	69.2	68.6	13.6
6.0	1 184	67.4	68.2	20.2	1 182	68.5	69.4	19.1

is that the reflex plane is divided by two intersecting line segments apparently the slope of the line segments and the point of intersection are determined by inspection not by any formal optimisation method. This makes statistical interpretation difficult.

Johnsen et al (1976) stated that ARTs for puretones in patients with cochlear deficits are largely independent of the audiometric threshold, and they queried the logic behind the Niemeyer Sesterhenn prediction formula However they showed a clear positive association between average hearing loss and the ART for white noise but they did not express this in terms of a linear regression equation They showed a scattergram for hearing loss against the difference between puretones and white noise ARTs which showed very little association It may therefore be inferred that inclusion of the puretone average ART in a prediction equation may not contribute to the prediction accuracy and may even degrade it.

Keith (1977) reviewed the work of Niemeyer & Sesterhenn and Jerger et al noting that audiogram slope prediction by reflex methods is equivocal and that prediction methods based on white noise reflexes were as accurate as those which also included puretone ARTs.

Hall (1978) compared two versions of the Jerger predictor with an unpublished regression predictor reported by Baker & Lilly (1976) The predictive accuracy of the three methods was similar and decreased systematically as a function of increasing age The [illegible] rger predictors was degraded in the presence of minor abnormalities of the tympanogram.

In a comprehensive report Jerger et al (1978*a*) examined the effect of age degree of hearing loss and audiogram configuration upon the ARTs to puretone and wide-band stimuli They found that all three variables influenced the reflex relations and that their effects were interactive Jerger et al (1978*b*) reported the effects of age on reflex prediction They found that predictive accuracy was substantially better in a group of children than for either young or old adult groups

Present study

We attempted to model existing and new schemes in order to evaluate the efficacy of ARTs in hearing threshold determination of a specific population—adults with presumed noise-induced hearing loss (NIHL) From this group 1207 patients referred to our clinic (MSH) for assessment between January 1975 and January 1978 with reliable puretone audiograms and bilateral reflexes to wideband noise were selected as subjects Data were analysed on the University of Toronto IBM 3033 computer primarily using the Statistical Package for the Social Sciences (SPSS)

Reflex measurement methods The ART measurements considered here were obtained using standard clinical methods Contralateral ARTs were obtained to tonal stimuli at frequencies of 250 500 1000 2000 3000 and 4000 Hz, and to unfiltered low pass and high pass filtered white noise The 3 dB point was 600 Hz for both filters. The units for the tonal

uating or comparing prediction formulae are far more comprehensive if the predicted quantity has ratio strength

The points made above and others may be applied to critical review of some of the major publications in this area

Niemeyer & Sesterhenn (1974)

The basic prediction principle was established by this brief but effective report The authors stated that the prediction formula was

$$ARTAV - PTAV = 2.5(ARTAV - ARTWN)$$

where ARTAV is the average ART to pure tones PTAV is the average pure tone threshold and ARTWN is the ART to white noise in dB HTL

The above form of the equation emphasizes the convergence of PTAV and ARTWN to ARTAV at different rates as PTAV increases It can be rearranged to

$$PTAV = 2.5ARTWN - 1.5ARTAV$$

Niemeyer & Sesterhenn stated this prediction equation without describing any formal optimisation method to derive it They also expressed their sample size in terms of ears rather than individuals This is a common practice the most obvious effect of which is to inflate sample size If estimates of variation of any auditory measure are based opon the number of ears alone then between-ear and between individual variation will be confounded

Niemeyer & Sesterhenn noted that prediction was less accurate if the hearing loss was sloping but they did not quantify this observation Since it seems unlikely that the slope of the hearing loss profile would be known in advance the relevance of a prediction equation restricted to flat losses is difficult to assess They noted that sloping hearing losses are associated with an increase of the ART for high pass filtered noise relative to that for loss pass noise but the relations were not quantified

Jerger and his colleagues (1974) in a large sample study gave considerable impetu to subsequent research on ART predictio methods They introduced the concept of predicting four categories of hearing loss severity utilizing an empirical rule based on Niemeye & Sesterhenn s work which appears t have strongly influenced subsequent thinking They considered this justifiable because th prediction is affected by the slope of the audiometric contour and because in a screening environment the accuracy associated with numerical prediction is deemed unnecessary Neither of these two reasons is sound An method of predicting categories of hearing los severity will be based upon decision rule utilizing the same underlying measures as numerical predictor Thus categorization w also be affected by the slope of the audi metric contour although it may not be s obvious due to the degradation imposed b the categorization Predictive errors which a called moderate by Jerger et al can be a large as 84 dB HL! (Johnsen et al 1976)

The prediction rule developed by Jerger al is based upon clinical experience and th results are expressed as a matrix of observe and predicted categories The observed coun or proportions in each of the matrix cells a only estimates of the true counts or proportions No information is given concerning th variability of these estimates So far as th present authors are aware this is also true all subsequent papers in which the data a categorized

Handler & Margolis (1976) partitioned hearing loss severity into two categories norm and abnormal Prediction is based upon th position of the patient s reflex measures in two-dimensional plane one axis of which is puretone ART the other a broadband/pur tone ART ratio Both dimensions contribute the separation between normal and abnorm groups This method makes explicit use puretone ART elevation in addition to th bandwidth effect Information is lost becau the hearing loss scale is merely dichotomize A further limitation of this graphical metho

Table IV *Pearson r values for various single ARTs Right ear*

ART type	0 5	1	2	4	WN	LPN	HPN
0 5 kHz	1 00	0 68	0 40	0.25	0 66	0 65	0.36
1		1.00	0 61	0 32	0.74	0.71	0 45
			1.00	0 49	0 61	0.55	0.54
4				1.00	0 41	0.37	0 51
WN			–		1 00	0 85	0 65
LPN			–			1.00	0 64
HPN							1 00

high-pass noise ART shows poorer correlation at low frequencies but has the highest association with 4 kHz of the three noise types

(*b*) *Relationships between ARTs and behavioural thresholds* Consider any pair of measures one of which is an ART the other a behavioural threshold. These can be represented on a bivariate plane such that observed values of these pairs will be points in the plane The pairs constitute a bivariate random variable which will be distributed over the plane

Some correlation coefficients for behavioural and acoustic reflex thresholds to pure tones are shown in Table V The ART to 2 kHz, for example, is correlated significantly with the PTT at that frequency However the correlation is extremely weak even though the relationship is highly significant statistically That is to say knowledge of either kind of threshold would give almost zero predictive power for the other kind of threshold This was true of all other PTT PT ART relationships studied

The correlations of noise ARTs and the PTTs are shown in Table V For the white noise stimulus the correlations are highly significant and fairly uniform over the frequency range For lowpass noise and highpass noise the trends are as expected the lowpass noise ART is more highly correlated with low stimulus frequencies and the highpass ART with high stimulus frequencies For the puretone average, the three noise reflexes correlate almost equally These correlations are among the highest in the table

(*c*) *Dependency versus prediction of PTT from ART* Since behavioural puretone thresholds are so fundamental it is natural to ask to what extent the various ARTs depend upon them. To answer this and simultaneously to examine in more detail the nature of the relationships which underlie the crude correlation coefficients the behavioural threshold must be treated as an independent variable and the ART as a dependent variable It is then necessary to examine the properties of the conditional distributions of the ARTs given various values of the behavioural thresholds The important point is that in studies of dependency it is the conditional properties of the ART which are examined

In the process of prediction of behavioural thresholds from ART measurements an entirely different view of the bivariate distribution should be taken. In this case it is necessary to regard the ART as the independent variable and the behavioural threshold as the dependent variable Predictive functions must be based upon the conditional properties of the behavioural threshold given values of the ART This sharp distinction is not usually brought out in reports concerned with hearing loss prediction from ART measures

These points are exemplified in Fig. 1 showing a cross-tabulation of the ART to white noise and the average PTT at 0.5 1 2

Table V *Pearson correlations of ARTs and behavioural thresholds Right ear*

ART type	Behavioural threshold frequency				
	0 5	1.0	2.0	4 0	AVE ½ 1 2, 4
0.5	0 07	−0.10	0.06	−0 05	0.05
1.0	0 13	0 07	0.05	0 01	0.09
2.0	0 10	0.10	0.20	0.14	0 18
4 0	0 01	0.02	0 08	0.22	0 11
WN	0 21	0.14	0 19	0.15	0..4
LPN	0 20	0 14	0.16	0.09	0.20
HPN	0.11	0 11	0.25	0.23	0.24

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Table II *Percentage of cases with measurable ARTs*

ART type	Right ear	Left ear
0.5 kHz	99 0	99.3
1 0	98.?	98 8
? 0	91 9	90 4
4 0	60.5	56.8
LPN	94.5	95 ?
HPN	86.9	84 9
WN	100 0	100 0

ARTs are dB HL re ANSI 1969 and dB SPL for the noise ART's

The reflex thresholds were measured on a Madsen Electro Acoustic Impedance Bridge Model Z0-72 1 Reflex responses were recorded on a Gould 2-channel strip recorder

RESULTS AND DISCUSSION

The selected sample comprised 1 207 cases mean age 54 9 years range 27–78 years

The puretone Air Conduction audiograms are summarized in Table I

In Table II the proportions of measurable reflex thresholds are shown It is clear that the proportion decreases monotonically with increasing frequency with a marked decrease at 4 kHz The proportions for noise ARTs are also shown Summary statistics for each reflex threshold are shown in Table III If the ART is greater than the upper limit of the measuring device it is ignored and the sample size changed accordingly From Table III it is clear that the bevaviour of the reflexes for the two ears is very similar The mean ART increases monotonically with stimulus frequency For the broadband stimuli the mean ART for LPN and WN are similar with that for HPN higher by about 7 dB All tonal ARTs show similar values of variability which are slightly more than those for noise ARTs It should be noted that the variability measured here is a combination of within subject and between-subject components presumably dominated by the latter

(*a*) *Relationship between tone and noise ARTs* A simple measure of the linear association between pairs of ARTs is the Pearson correlation coefficient Significance tests are exact only if the underlying variates are bivariate normal which is not the case here. However inspection of the various *r* values shown in Table IV permits qualitative statements about relative strength of associations. All coefficents are highly significant statistically and all associations are positive For the puretone ARTs the coefficients decrease as the frequency separation increases as is expected

The white noise ART is strongly correlated with all puretone ARTs with a maximum at 1 kHz and a decline at 4 kHz. The low pass noise ART behaves similarly but with a greater decline as frequency increases The

Table III *Summary statistics for ARTs*

	Right ear				Left ear			
ART type	*N*	Mean	Median	S D	*N*	Mean	Median	S D
0 5 kHz	1 196	87 6	87 1	8 0	1 199	87 4	86 6	7 9
1 0	1 186	90 9	90 4	7 2	1 194	90.8	90 4	7.3
2 0	1 110	94 8	94 3	7 3	1 09?	95 1	94 6	7.5
4 0	731	99.3	99.5	7.5	686	99 6	100.2	7 8
LPN	1 142	93 4	93.3	6 7	1 150	93 7	93 7	6.9
HPN	1 050	100 4	100 5	5.8	1 025	100.9	101 1	6 1
WN	1 207	93 5	93..	6 4	1 207	93.8	93.5	6 6

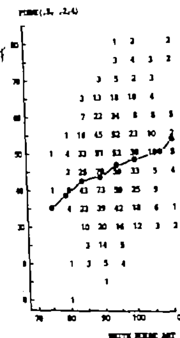


Fig. 3 Cross-tabulation of average ART at 0.5 1 2 and 4 kHz and the white noise ART. Circles are the conditional mean average PTT

and thereafter shows a rapid rise. The white noise ART increases fairly linearly throughout the loss range. The convergence principle is therefore valid for losses up to 70 dB. These results are comparable with those obtained by Jerger et al. (1978*a*)

(*e*) *Prediction of audiogram features from ART measurements.* Most published reports on prediction of hearing level from ART measurements consider average hearing losses. Such an approach arises from an historical perspective or because of an underlying presumption that average hearing losses will somehow relate better to ARTs than will single puretone hearing loss measurements. The results in Table V show that this is not entirely true but the noise ART/average loss correlations are among the highest in the table.

The predictability of the average puretone loss at 0.5 1 and 4 kHz by single ARTs was examined. From Table V it can be seen that the white noise and high pass noise ARTs correlate equally well. The former was

Table VI *Conditional statistics analysis of variance and regression data for the average PT threshold at 0.5 1 2 and 4 kH by ARTWN*

PTAVE statistics

ART value	Mean	S D	*N*
75	34 4	1..5	6
80	39.0	10 7	23
85	42.8	9 1	165
90	43.8	10.3	357
95	46.3	10.6	350
100	47.9	11.0	186
105	49.5	10.5	80
110	53.9	14.2	25

Analysis of variance:
Between groups. p less than 0 0001
Linearity p less than 0 0001
Nonlinearity NS

Simple linear regression:
PTAVE=7 6+0.4 ARTWN

Standard error of slope: 0.048.
Residual mean square: 109 7
F ratio 72.9 p less than 0 0001
Number of cases: 1192.

selected for detailed study because of its higher incidence (Table II)

The cross-tabulation of the average behavioural threshold at 0.5 1 2 and 4 kHz versus the white noise ART is replotted in Fig. 3 with the white noise ART as the abscissa. It can be seen from Fig 3 that the conditional mean average loss increases linearly with increased ART (conditional statistics and regression data are given in Table VI) The key observation is that the range of the conditional means and therefore the approximate range of prediction, is only about 20 dB and the standard error of prediction is greater than 10 dB at any value of white noise ART. Therefore although the white noise ART does permit prediction of average hearing losses, the clinical utility of this prediction is extremely limited

Clearly the conditional average loss distributions will depend upon the population under study. If this were restricted in range then the range of prediction would also be restricted. We do not consider this to be a serious objection to these data. The range of average losses

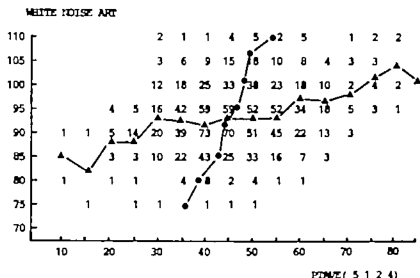


Fig 1 Cross-tabulation of the white noise ART (dB SPL) and the average audiometric threshold at 0.5 1 and 4 kHz The latter is rounded to 5 dB steps Triangles are conditional mean ARTs (column means) circles are conditional mean PTTs (row means)

and 4 kHz. These two variables form a bivariate plane quantised into 5 dB squares The cell frequency is the number of times that co-ordinate pair was observed If these were divided by the total number of observations then the height of each cell proportion above the bivariate plane would estimate the joint probability function There is a tendency for large values of the white noise ART to be associated with large values of average hearing loss and vice versa This is the reason for the highly significant positive *r* of 0 24 shown earlier in Table V For dependency of the white noise ART upon the average hearing loss a natural statistic is the conditional mean ART for each column of the figure These conditional means are indicated by triangles in the figure It can be seen that there is a slight but definite trend towards increasing ART as the average hearing loss increases

For prediction of average hearing loss using the white noise ART the appropriate measures are the conditional means of the behavioural thresholds for each row of the figure that is for each value of the white noise ART Such means are indicated as circles in the figure It is extremely important not to confuse these two aspects of bivariate distributions Not only are the conditional mean curves required in each case entirely different but it is perfectly possible that while good dependency may exist predictive relationships may be poor and vice versa

In this report therefore dependency and prediction will be treated as entirely separate issues with major emphasis on prediction for the dependency of ARTs on PTTs has been commented upon by other authors and our findings are similar

(d) Dependency of ARTs on average hearing loss The basic rationale proposed by Niemeyer & Sesterhenn is convergence of broadband and average tonal ARTs as the hearing loss increases This concept is valid for our data as shown in Fig 2 This figure shows the conditional means for the average tonal ART at 0 5 1 and 2 kHz and the white noise ART as a function of increasing average hearing loss at 0 5 1 2 and 4 kHz The average ART is constant until a loss of 70 dB

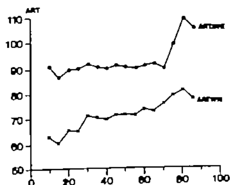


Fig 2 Means values of ART against average PTT at 0.5 1 and 4 kHz Circles are the average ART at 0.5 1 and kHz. Crosses are the white noise ART

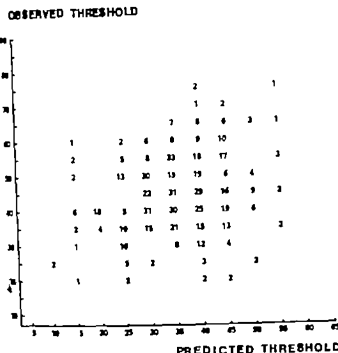


Fig 5 Cross-tabulation of observed average PTT (ordinate) predicted average PTT (abscissa) from the Niemeyer Sesterhenn predictor

Comparative assessment of predictive formulae

The predictive performance of the Niemeyer–Sesterhenn formula was compared with prediction using white noise alone and with a multiple regression equation derived in this study The Niemeyer–Sesterhenn formula incorporates the 4 kHz ART Accordingly the formulae were compared in a sub-population of 719 patients having all appropriate reflexes

In Fig. 4 the difference between the average ART at 0.5 1 2 and 4 kHz and the ART for white noise is cross-tabulated against the average ART minus the average puretone threshold at those frequencies. This corresponds to Fig. 3 in Niemeyer & Sesterhenn's original report (1974)

The solid line in Fig. 4 corresponds to their prediction equation which states that the abscissa is 2.5 times the ordinate The data of this study do not follow that relationship the bivariate distribution is clearly more horizontally oriented than that required by the formula A substantial number of points are outside the 20 dB limits especially for small values of the abscissa.

One way of visually assessing the performance of a prediction equation is to cross tabulate the observed and predicted values In Fig. 5 this coss-tabulation is shown for the Niemeyer–Sesterhenn predictor If the predictions were perfectly accurate all the points would lie on a diagonal line with unit slope The enormous scatter is clear from the figure There is a tendency for predictive errors to be positive at low predicted values and negative at high predicted values As well as a great deal of variation there appears to be systematic bias in prediction. These results differ in both location and scatter from those of Miller et al. (1976) but that study is both small-sample and contains a normal-hearing group

Carrying out a simple linear regression of the four-frequency average hearing loss on the white noise ART in this sub-population gave a predictive formula shown in Table VII the correlation coefficient was 0 22. The cross-tabulation of observed and predicted values is

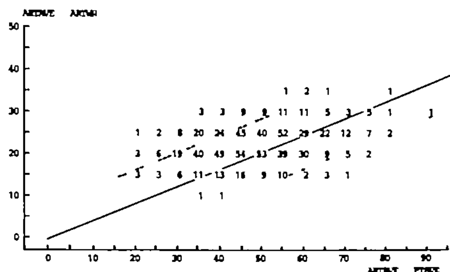


Fig 4 Cross-tabulation of the components of the Niemeyer-Sesterhenn predictor ARTWN is in dB HTL. The solid line represents perfect prediction The dotted lines are 20 dB bounds on predictive error

is from 10 to 85 dB and there is ample freedom within the data for a good predictive relationship to show itself It does not the main feature of the data being the enormous range of average losses observable for any given ART

The results of this study were compared with those of Johnsen et al (1976) Fig 3 is analogous to the scatterplot of Johnsen et al (in their Fig 3) There are several remarkable differences between the two sets of results The most obvious is that they obtained a correlation coefficient of 0 88 whereas that obtained in this study is 0 24 This is due to the rate of change of average hearing loss for a given change in ART being much greater in their study than in this one The ranges of ART in the two studies are comparable and if for example the range from 75 to 110 dB is taken then Johnsen et al obtained a range of conditional mean from approximately 15 to 85 dB that is a 70 dB range In the present study the range is from approximately 35 to 55 dB only a 20 dB shift

Johnsen et al have extended the range of ART measurements by incorporating the results from a normal hearing group Such a procedure is inappropriate in prediction for two reasons Firstly as previously mentioned prediction is a process of producing conditional statistics for hearing thresholds from ART measurements If a normal-hearing group is included then clearly there has been some kind of selection process of the permissible value of the average hearing loss for that group The conditional statistics are therfore biased Secondly an unevenly sampled range of abscissa values can lead to the classic hole in the middle error in regression analysis

It is clear from the data of Johnsen et al. that even if the normal group is excluded the correlation coefficient will be substantially higher than that obtained for this study A possible reason is that the sample sizes at 105 and 110 dB in the Johnsen et al study are extremely small compared to the present study Any correlation or predictive function produced by the two studies will be more reliable for that with the larger sample size, that is confidence intervals on estimates will be smaller There is also the possibility that a large sample study will naturally reflect more variability sources than a small one and that this will better reflect the variation in the population at large Furthermore the nature of the patient populations in the two studies may differ In the present study the aetiology is overwhelmingly NIHL but Johnsen et al did not specify the aetiology of hearing loss in their patients Perhaps ART PTT relationships are dependent upon subtle variations in pathology within the group of sensorineural hearing loss patients

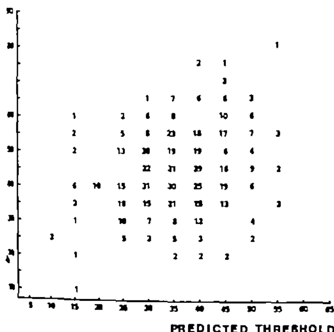


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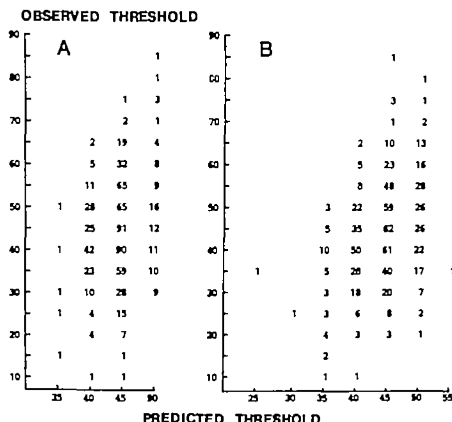


Fig 6 Cross-tabulation of observed (ordinate) and predicted (abscissa) average PTT for (A) linear regression on white noise ART and (B) multiple linear regression on white noise and 500 Hz ARTs.

shown in Fig 6*a* Although there is no obvious bias the scatter is comparable to that of the Niemeyer–Sesterhenn formula and the predictive range is very restricted

Applying multiple linear regression in this population and allowing the ARTs to white noise 500 Hz 1 kHz and 2 kHz as possible independent variables led to an equation involving only the white noise and the 500 Hz reflexes This equation is shown in Table VII This gave the best correlation coefficient of all the predictors discussed here

The cross tabulation is shown in Fig 6*b*

Table VII *Comparison of predictive formulae in the subpopulation with measurable ARTs at 0.5 1 2 and 4 kHz*

Niemeyer–Sesterhenn PTAVE=2.5 ARTWN−1.5 ARTAVE	=0.24
MSH data. White noise ART regression PTAVE=0 43 ARTWN+5	=0.[illegible]
MSH data. Multiple regression on WN 0.5 1 2 kHz ARTs PTAVE=0 81 ARTWN−0 45 ART500+9	=0.34

There is no obvious bias but the range is almost as severely restricted as for the white noise predictor The standard error of prediction is still greater than 10 dB

It should be noted that the Niemeyer-Sesterhenn formula is not a regression formula and therefore not subject to the constraint of producing the minimum mean squared predictive error Such unconstrained formulae can be made to produce any desired range by simply adjusting the multiplicative constant. The occurrence of a good predicted range for any formula is only a positive attribute if other features such as bias or scatter are not compromised

No matter what set or reflex measures is used to predict the puretone hearing loss, linear combinations of these measures do not contain substantial predictive power Even the best of them that is the multiple regression formula using the white noise and 500 Hz reflexes with the correlation coefficient of 0.34 only suffices to explain less than 12% of the observed variation in average hearing loss Although highly significant statistically this is

Table VIII. *Regression data for low and high slope groups*

White noise ART alone			
All	PTAVE= 7 6 0 41 ARTWN	r=0 .4	N=1 192
Low slope	PTAVE −15 4+0 67 ARTWN	0.33	N= 289
High slope	PTAVE 15.5+0 30 ARTWN	=0 20	N= 389
Multiple regression on ARTWN 500 1K 2K			
All	PTAVE 16+0.73 ARTWN−0.45 ART500	=0.33	
Low slope	PTAVE −1 4+0 87 ARTWN−ART500	=0.32	
High slope	PTAVE= 29−0 42 ART1K+0 40 ART2K	r=0.32	

really very poor performance for a predictive function

As Jerger's categorical analysis has been widely discussed the predictions of these various formulae were subjected to such an analysis using his categories The errors of loss prediction by the Niemeyer–Sesterhenn formula the white noise regression formula and the multiple regression formula were compared with those achieved by Jerger et al (1974) and the results are shown in Table IX

In view of the preceeding discussion of the limitations of ART based predictors it seems that categorical analysis is misleading for the population under study

The effect of audiogram slope on prediction

In the original report by Niemeyer & Sesterhenn and in several subsequent reports it has been stated that the predictive accuracy of acoustic reflex measurements is less in high slope audiograms This assertion has not been fully quantified in the published literature

Two groups of patients were extracted from the overall population Audiogram slope was defined as the difference between the puretone thresholds at 4 kHz and 1 kHz. There were 289 low slope (10 to 25 dB) and 389 high slope (40–55 dB) patients

The best single predictor variable in the whole population was the white noise ART (Table VI) The results of applying linear regression separately to the low slope and high slope groups are shown in Table VIII The regression and ccorrelation coefficients are quite different for the two groups. Conditional means and regression lines are shown in Fig 7

These data show that the accuracy of prediction of average hearing loss by the white noise ART is indeed affected adversely by audiogram slope The difference between the regression coefficients for the two slope groups is disturbing since it means that the population is highly heterogeneous with respect to the predictor function that should be used The prediction function for the whole population is clearly a compromise between that for the low slope and high slope groups and is not particularly appropriate for either group

Table VIII shows the result of applying

Table IX *Comparison of errors for various prediction methods*

Error (%)	Niemeyer Sesterhenn	White noise regression	Multiple regression	Jerger et al. (1974)
None	58	6.	63	60
Moderate	41	38	37	36
Serious	1	0	0	4

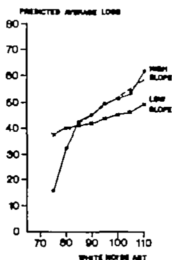


Fig 7 Conditional mean average PTT for low and high slope groups against the white noise ART The dashed and dotted lines are regressions

multiple regression analysis to the low and high slope groups independently It can be seen that the regression equations for the two groups are highly dissimilar to the extent that they do not even contain the same variables! It is interesting to note that the stepwise algorithm has not even selected the white noise ART for inclusion in the regression formula for the high slope group It should be noted that the correlation coefficients are 0.37 for each group which compares very well with the 0 33 for the overall population This is an interesting result implying that it is possible to achieve a similar degree of predictive power in both groups but that the predictor functions

Fig 8 Estimated cumulative distribution functions for the PTT at 2 kHz ART takes the decade values indicated. The starts indicate the PTT which is exceeded in 10% of cases.

necessary to achieve this involve completely different sets of variables.

The decreasing effectiveness of the white noise reflex as a predictor of average hearing loss in the high slope group and the production of completely different multiple regression predictor equations for the low slope and high slope groups, make the use of ART prediction procedures even more problematic

One possible solution to this is to develop prediction procedures in two stages the first consisting of the prediction of slope and the second a prediction of severity To this end the ability of reflex measures to predict audiogram slope was examined The measure selected was the ART to high pass noise minus the ART to low pass noise This was both correlated and cross-tabulated against the observed audiogram slope as measured by the difference between the 4 kHz and the 1 kHz puretone thresholds The correlation coefficient obtained was only 0 22 and the cross-tabulation showed no appreciable diagonalization. It is concluded that this ART measure cannot serve as a useful basis in a multistage prediction process

One final and simple examination undertaken attempted to relate values of PTT to ARTs for PTs It has often been pointed out that if a recorded PTT were worse than a measured ART for that frequency the PTT must be in error (Alberti 1970) However the literature is not clear how much higher the ART must be than the PTT before the latter can be considered reliable We investigated what level of PTT had a less than 10% chance of actually being exceeded for different decade levels of ART The results for 2 kHz are shown in Fig. 8 where it can be seen that if the ART is 100 dB or greater the PTT has a 90% probability of being at least 35 dB better whereas if the ART is 90 or 80 then the PTT may come to within 25 dB of the ART If it comes closer the patient should be re examined audiometrically before accepting the result

The results of this study are disappointing. In adults objective predictive techniques are potentially of greatest use in medico-legal settings, particularly with large populations such as in screening industrial hearing loss claims It is exactly such a population which has been tested and where all simple techniques appear to have no real quantitative value ART measurement has a qualitative role to play both in site of lesion testing and in identification of hearing loss but no accurate quantitative predictions about hearing loss can be based upon it

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Dr P W Alberti
Suite 405 600 University A enue
Toronto Ontario
Canada M5G 1X5

Acta Otolaryngol 89 358–361 1980

THE CRITICAL INTENSITY FOR OCCUPATIONAL NOISE

F Schwetz U Doppler R Schewczik and B Welleschik

I HNO-Univers Klinik Wien Austria

Abstract Decades of medical examinations have shown that large-scale risks of hearing defects exist not only from 90 dB(A) upwards but even from 85 dB(A) upwards This experience is confirmed by the analysis of 25 000 tone threshold audiograms and by the results of experimental hearing fatigue tests

The critical intensity is of interest not only audiologically but also from the viewpoint of occupational medicine As is generally known the noise to which people are exposed at their working places leads at first to a high tone hearing defect (Fig 1) and later to a reduction in language hearing Thus a certain proportion of persons exposed are affected by noise induced hearing defects

Such hearing defects develop when the work noise exceeds the so-called critical intensity If the noise level is lower the hearing remains intact despite several hours of daily work This fact explains the intensive endeavors aimed at finding a generally applicable value for this critical intensity

For two reasons this has been difficult 1) As opposed to other work related organic defects the human ear shows highly variable sensitivity to noise influences depending on the respective person 2) so far no hearing measurement results have been gained in large scale examination series nor have regular surveys of technical noise data been carried out at the work places But it is only in this way that the hearing defects and hearing levels can be correlated

The intensities referred to in medical literature are thus not based on the evidence of actual hearing losses but rather on the results of experimental hearing fatigue tests Accordingly the data vary between 70 and 105 dB Only recently have we realized that the actual value must lie between 80 and 90 dB(A). Although this margin amounts to only 10 dB it gives rise to two important considerations.

1) *An economic consideration*

In a large number of enterprises the noise level lies between 85 and 90 dB(A) If the critical intensity is fixed at 90 dB(A) this means a marked reduction of the costly supervision of hearing

2) *A medical consideration*

The economic requirement must be met only if there is medical proof for the fact that noise levels cause work-induced hearing defects only upwards of 90 dB(A)

Series of audiometrical examinations in workers exposed to noise have been carried out in Austria since about 1957 Very early I made the observation that noise levels of below 85 dB(A) cause parctically no hearing defects while noise levels of 85 dB(A) cause the same defects as those of 90 dB(A) For this reason I recommended many years ago that the lower limit of the critical intensity be fixed not at 90 but at 85 dB(A) In Austria the audiological examination program is based on this value as its guideline

Recently two projects were carried out at the audiology department of the First Oto-laryngological University Hospital Vienna They confirm the pathogenicity of the 85 dB(A) noise level

Sound-audiometrical measurement results obtained in about 25 000 workers exposed to different noise levels were subjected to analysis (Fig 2) There are five groups of noise levels. below 85 dB(A) 85–88 dB(A) 88–91 dB(A) 91–97 dB(A) and over 97 dB(A)

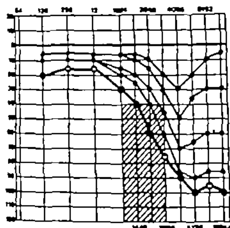


Fig 1

The study covers hearing losses at the most severely affected frequency of 4 kHz and such at the frequencies of 3 and 2 kHz. The calculations are made not on the basis of the arithmetic mean value but in percentiles. The breakdown of the data permits a listing of the hearing loss in the individual frequency ranges suffered or exceeded by a certain percentage of test persons. A percentile of 50 indicates that 50% of the random samples are below and 50% over this point. A percentile of 90 means that 90% of the hearing losses covered are below this value and only 10% are above. It is above all the hearing losses suffered at 4 kHz which are of interest. As can be seen from the 50-percentile curve there is a slight increase at the 85 dB(A) level levelling off significantly toward 91 dB and even 97 dB. The hearing loss curve rises again more markedly only from a noise level of 97 dB upwards. This tendency can be seen even better in the 90-percentile curves. In line with the typical noise induced hearing loss the situation is similar at a frequency of 2 kHz, there are few changes however in the hearing losses suffered at 2 kHz. According to the results of this study the hearing defect break is to be found not at 90 but at 85 dB(A). The practical implications of this fact are seen in our own data material. Out of about 25 000 persons covered 23 446 are exposed to a noise level of 85 dB(A) and more (Fig. 3). Of them 59.6% work at noise levels lying between 85 and 90.6 dB(A). If in their case 90 dB(A) had been fixed as the decisive value for audiometric supervision a majority of them would not have been medically covered at all despite the same hearing risks.

Our second consideration relates to experimental studies. Today it is generally recognized that repeated noise induced hearing fatigue is responsible for permanent hearing

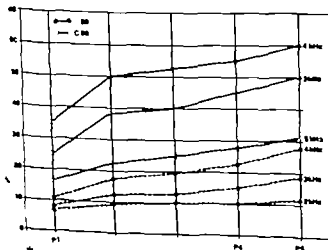


Fig 2 Hearing losses and exposure intensity

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2) The analysis of 25 000 tone threshold audiograms made after hearing losses and noise exposure confirms the data gained in practical work.

3) Experimental studies show—as a consequence of the 85 dB(A) noise level—a lack of hearing recovery which is the ultimate cause of the permanent hearing defect

ZUSAMMENFASSUNG

Wie die jahrzehntelange ärztliche Begutachtung zeigt, bestehen stärkere Hörschädigungsgefahren nicht erst ab 90, sondern schon ab einem Lärmpegel von 85 dB(A). Diese Erfahrung wird durch eine Analyse von 25 000 Tonschwellenaudiogrammen und durch das Ergebnis experimenteller Gehörermüdungstest bestätigt.

F Schwetz
I HNO-Univ.Klinik, Wien
A-1090 Lazarettgasse 14
Austria

DISCUSSION

Spoendlin to Schwetz: Your first slide showed that there very little barely significant increase of damage risk between noise levels of 85 and 90 dB(A) whereas much greater increases exist between 75 and 80 dB(A) or 90 and 95 dB(A) What therefore is the reason t propose 85 dB(A) as permitted noise level limit rather than 90 dB(A) as it is in Germany and Switzerland? The barely significant increase in damage risk will probably not justify the enormous costs of reducing industrial noise emission by 5 dB

Tonndorf to Schwetz. W in the US would also be happier with 85 dB(A) maximal level However as you are fully aware this is not a scientific but a political decision At the international level at the ISO (w ll have meeting in Delphi? Sept. 26) I am happy to report (1) that the new standard will no longer give fixed values like 90 dB(A), but only guidelines so as to force the administrative authorities *and into action, (2) the three* speed frequencies will be increased to four 5 1.0 2.0, 3.0 HHZ which will make the index more sensitive

Schwetz (Reply).
The contributions made to the discussion, for which I am most grateful can be answered together

Of course the problem of fixing the critical intensity value at certain point is also a political question in that it touches pon the financial aspect But as it involves the question of maintaining the health of the working population, it must be seen first and foremost as medical one I too encountered difficulties with the competent authorities whe many years ago I wanted to introduce 85 dB(A) as the critical intensity value to be generally observed in Austria what I demanded at that time was based only on individual observations! The results which are now submitted *should make it easier* for the medical profession to convince the politicians through factual material

As concerns the effect of noise-induced damage on sound-hearing, let me say the following: For the maintenance of speech hearing neither the auditory threshold *for 3 kHz nor that for 2 kHz is of any decisive significance.* As I was able to show only recently on the basis of larger number of patients examined, the speech discrimination capacity depend above all on the auditory threshold loss at 1.5 kHz in the case of typical noise-induced damage

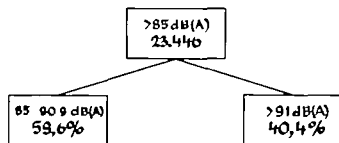


Fig 3

defects Therefore a study was carried out to establish the extent to which different noise levels can influence the recovery of hearing after a person s exposure to noise

Two groups of young persons with normal hearing were exposed to different noise levels At 3 kHz the first group showed a tone threshold shift (TTS_2) of 32.5 dB (Fig 4) the other of 17 1 dB During the phase in which the recovery of hearing commences the ear is exposed again to noise of different secondary levels As concerns our problem the noise levels of 75 and 85 dB are of main significance Their influence on the tone threshold curve is observed over a period of 2 hours When the TTS_2 was higher (32 dB) the hearing recovered rapidly in the first hour during the 75 dB noise only in the second hour does it show some delay The 85 dB noise however effects from the very beginning a smaller degree of recovery which finally comes to a stop and in the second hour is followed by yet another hearing fatigue

If the initial value of TTS_2 is not so high (about 17 dB) the different effect of the two secondary exposures to noise is even more pronounced Even if much delayed signs of hearing recovery during the 75 dB noise the 85 dB level however prevents any recovery effect and even further intensifies the hearing fatigue

Even if the effects of 85 dB noise on the unexposed organ of hearing are left out of consideration these results nevertheless suggest the following An ear fatigued by noise exposure not only gives up the chance of recovering its hearing faculty but finally suffers from an even more severe hearing fatigue. This seems to explain why an increasing number of permanent hearing defects are found upwards of this noise level

By way of summary we may state as follows

1) For many years medical examinations have shown that even noise levels of 85 dB(A) upwards cause marked hearing defects partly even severe ones In addition differences in the hearing loss cannot be found to exist in such persons when compared with persons exposed to somewhat higher noise levels

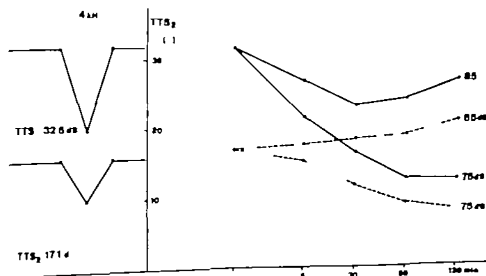


Fig 4

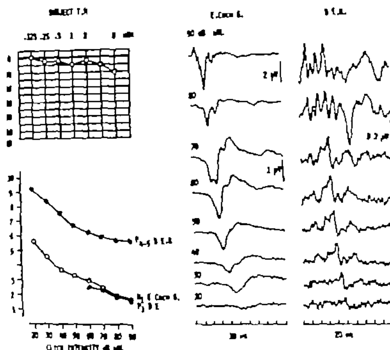


Fig 1 Typical results obtained with ECochG and BER in one normal subject. In these and all subsequent traces negativity at the active electrode is plotted downwards.

n conjunction (Coats & Martin 1977 Salomon & Elberling 1979) the combined use of ECochG and BER is not a common procedure in the clinic

This paper reports the first results we obtained with the combined use of ECochG and BER in the differential diagnosis of cochlear/retrocochlear disorders Certain limitations of both methods are shown and the importance of their conjunction is emphasized

METHODS

Forty-nine subjects were tested in this study Nine of them were normal-hearing and constitute the normal group Among the other 40 presenting with various degrees of hearing impairment a central tumour (generally acoustic neuroma) was confirmed by X-ray and surgical operation in 14 cases which constitute the 'retrocochlear group the remaining 6 patients, mainly Menieres, showed no sign of retrocochlear disorder and were ranged in the cochlear group

For TT ECochG the classical electrode positions were used the active electrode rested on the promontory a reference disc electrode was placed on the earlobe and the patient was grounded with a disc electrode on the forehead For BER recordings small needles were inserted under the skin at the vertex (active electrode) and in the ipsilateral mastoid region (reference) the ground electrode remained as before on the forehead

A broad-band click was used for acoustic stimulation it was alternated in polarity and presented at a rhythm of 10 per second for ECochG and 70 per second for BER, it was delivered either in free field through a loudspeaker (Altec 808-8A) for ECochG or in close field through an earphone (TDH 49) for BER Moreover for brainstem recordings a wide-band masking noise was sent to the contralateral ear through an earphone at about 30 dB below the click level in the test ear in order to avoid crossing over of click stimulation In the majority of the patients in this study ECochG and BER were performed successively and click-evoked responses ...

Acta Otolaryngol 89 362–369 1980

TRANSTYMPANIC AND SURFACE RECORDINGS IN THE DIAGNOSIS OF RETROCOCHLEAR DISORDERS

M Portmann Y Cazals M Negrevergne and J -M Aran

F om Clinique Universitai e d'ORL et Laboratoire d'Audiologie Expérimentale (FRA Inserm no 39) Bordeaux France

Abstract A series of patients presenting with subsequently surgically confirmed central tumour involving the auditory pathways were investigated using both transtympanic electrocochleography (TT ECochG) and surface recordings of brainstem evoked responses (BER) While ECochG allows a detailed study of peripheral function BER allow the investigation of neural conduction up to the level of the inferior colliculus Valuable information can be obtained from (1) comparison of the amplitudes of the sensory and neural components of the ECochG (?) comparison of ECochG BER and aud tory thresholds (3) time interval measurements between the auditory nerve response (N on the ECochG) and the inferior colliculus (wave P_{4-5} on BER) (4) contralateral comparisons Similar measurments performed in pure cochlear pathologies mainly in Meniere s disease yielded very significant differences

The development of transtympanic electrocochleography (TT ECochG) in the clinic during recent years has demonstrated that it can give much precise information on the functioning of the end organ (Aran 1973 Eggermont 1976) However when information is needed on the condition of the auditory system beyond the cochlea TT ECochG is of limited value Some characteristics of ECochG responses have been related to the existence of retrocochlear disorders by several authors (Portmann & Aran 1972 Brackmann & Selters 1976 Odenthal & Eggermont 1976 Gibson & Beagley 1976) they are mainly a distorted pattern of the response a shifted latency/amplitude function and a threshold discrepancy with subjective hearing However none of the proposed criteria allows a systematic detection of retrocochlear impairment only a small proportion of these pathologies being thus diagnosed

More recently sound-evoked responses from the brainstem (BER) were shown to be recordable from surface electrodes and, although the responses are of low amplitude, some of their characteristics can be reliably measured even at low intensities The study of retrocochlear disorders with BER has received much attention during recent years As a first step several measures of amplitude and/or latency of the various waves of BER have been proposed (Sohmer et al 1974 Thorton 1975 Starr & Achor 1975) and related to a variety of pathologies However among the latest studies the latency of the most prominent waves the fourth and fifth peaks (P_{4-5}) seem the most reliable and significant measure (Selters & Brackmann 1977 Stockard & Rossiter 1977 Clemis & Mitchell 1977 Coats & Martin 1977 Terkildsen et al 1977 Rosenhammer 1977) On the basis of BER recording alone a high proportion of retrocochlear disorders was reported to be detectable as producing a significant delay in the latency of the P_{4-5} wave However the values of P_{4-5} latency are closely dependent upon the condition of the peripheral organ and latency shifts attributable to cochlear pathologies are often undiscernible and can lead to misinterpretations (Clemis & Mitchell 1977 Coats & Martin 1977 Brackmann & Selters 1979 Salomon et al 1979) To avoid such difficulties combining of TT ECochG with BER recordings is appropriate since it gives precisely the latency of the neural activity at the cochlea level Although both techniques give complementary information and appear more efficien

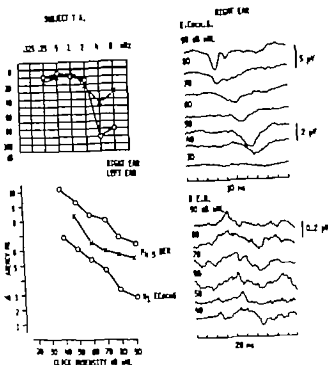


Fig 3 Typical example of the effects of serious high-frequency hearing loss on BER responses Compare the results with those in Fig 2.

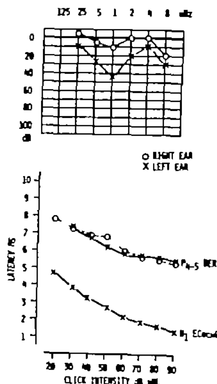
measures to latencies. In Fig. 1 are presented the results from one normal subject In all normal cases the latency/intensity curves of ECochG peak (N) and BER wave P_{4-5} were parallel and showed a latency difference of about 4 ms (see also Fig. 5) At high intensity levels the latency of the first peak (P) in BER could be measured and was found to correspond to that of ECochG N but for middle and low intensities it was impossible to detect.



Fig 4 Result from Meniere patient presenting rith recruitment. Although the threshold is elevated the first peak (P) in BER recordings appears clearly

Cochlear/retrocochlear cases

On the basis of ECochG results alone we found that the strongest indicator of a retrocochlear disorder was a threshold level better than could be expected from the subjective hearing revealed by the audiogram. Statistical studies relating the click threshold at ECochG to the subjective audiogram showed that it is a good indicator of subjective audiometric thresholds for middle frequencies (Eggermont, 1976) In all the subjects in this study we compared the ECochG click threshold with the best audiometric threshold for frequencies of 1 2 and 4 kHz. The ECochG click threshold was better in 4 out of the 14 patients with a confirmed tumour such a phenomenon was never observed in the group with cochlear impairment or in the normals. The pattern of ECochG response in itself did not appear sig



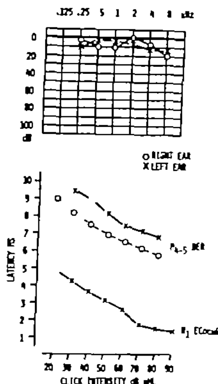


Fig 2 Comparison of ECochG and BER results in patients presenting with normal hearing or a moderate loss In the case of unilateral neuroma, note the increase in latency of the BER response from the affected side

tained from high intensities down to threshold in 10 dB steps

A few cases were investigated with more sophisticated equipment allowing simultaneous ECochG and BER recordings using a multichannel system In these simultaneous recordings we found it very interesting to use our stimulating paradigm earlier developed for the study of adaptation in ECochG Trains of five clicks with an interclick interval of 10 ms thus stimulating at rhythm of 100 per second are presented every 100 ms i e at a rhythm of 10 per second For ECochG this technique apart from the study of neural adaptation offers the possibility to precisely evaluate the sensory component (summating potential) of broad and abnormal responses For BER adaptation can also be studied and in the same time five BER traces are obtained which can be compared in order to improve the detection of a response if needed In these simultaneous ECochG and BER recordings clicks were delivered in free field by a loudspeaker and contralateral masking was applied through an ear phone

In all conditions successive or simultaneous recordings the patients were slightly sedated with a Nembutal suppository at a dose of about 2 mg/kg in order to improve the quality of BER recordings

RESULTS

Normal subjects

In the group of normals both ECochG and BER responses could be recorded down to very low intensities close to the normal hearing threshold for the click (see Figs 1 and 8). For ECochG responses were always very clear and both the amplitudes and the latencies could be reliably measured at all intensity levels and the waveform was easily recognizable In BER recordings the series of waves appeared clearly at the highest intensities 70 to 90 dB above threshold but at middle or low intensities only one peak remained detectable that corresponding to the fourth and fifth waves (P_{4-5}) We found that due to the poor signal to noise ratio amplitude measurements were not reliable and therefore we limited our

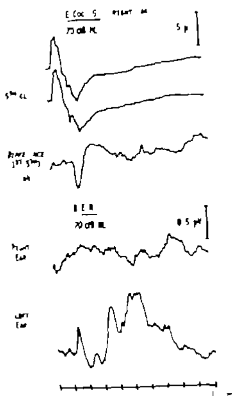


Fig 7 ECochG and BER responses in patient with neurinoma on the right side Note that the ECochG -eals clear response of abnormal pattern from the right -des and the evaluation of the neural and sensory components -th the study of adaptation In BER traces no -ponse at all could be recorded from the right side -ereas normal responses were clearly seen from the affected side

(the patients presented recruitment and at -gh intensities P could be observed in 13 out of the 26 cases such an example is shown in Fig. 4 Only for those 13 subjects could the latency interval between the cochlear nerve response (P) and the late brainstem potential P $_{\cdot 5}$) be measured with BER alone

Therefore in the majority of pathological cases ECochG was necessary to evaluate the results of BER, as it permitted one to calculate the latency interval between the peripheral and brainstem responses This time interval between ECochG N and BER P_{4-5} was found to be a very good criterion to differentiate between cochlear and retrocochlear disorders

Fig 8 BER ECochG click threshold differences as observed in all the subjects in this study Note that the results in normal and cochlear groups are concentrated in most cases around 0–20 dB In contrast, noticeable proportion of retrocochlear cases presented significant threshold difference

as shown in Fig 5 All data points obtained for all subjects are presented in this figure and it can be seen that the latency interval is constant at about 4 ms for the normal and cochlear groups, whereas in cases of retrocochlear disorders it is increased We found that a value of 4.5 ms was the best limit to distinguish cochlear from retrocochlear disorders In 3 patients with a tumour we observed at some intensities a latency interval below the 4.5 ms limit in such cases we considered the results over several intensities and found that for each patient the majority of values exceeded the 4.5 ms limit. These examples are shown in Fig. 6*a* Using this criterion 2 cases one cochlear and one retrocochlear were misclassified Their results are presented in Fig. 6*b* The case of confirmed tumour presented an ECochG click threshold better by about 25 dB than could be expected from the audiogram which is a strong indicator of a retrocochlear impairment The case of cochlear pathology was identified as a Meniere and presented no other sign of retrocochlear impairment.

In 4 patients of the retrocochlear group we could clearly record an ECochG response on the affected side whereas no response at all could be observed on BER recordings In these subjects we could verify that normal BER waves were recordable when stimulating the unaffected side Such an example is pre

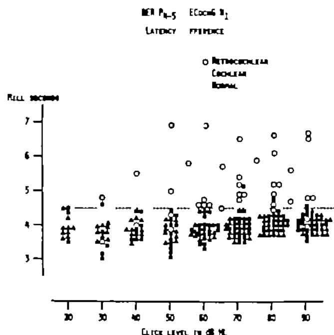


Fig 5 Latency interval between BER P_{4-5} and ECochG N All data from all subjects are reported Retrocochlear disorders clearly induce an increased latency interval The dotted line represents the 4.5 ms limit separating at best retrocochlear cases from either cochlear pathologies or normals

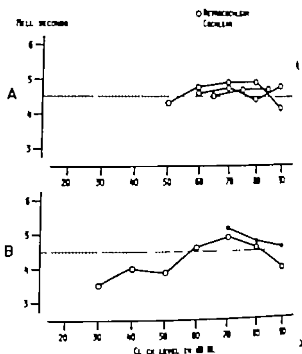


Fig 6 BER P_{4-5} – ECochG N latency intervals for certain particular subjects (A) Subjects from the retrocochlear group who showed some values of latency interval below the 4.5 ms limit at some intensities. In each case, however most values exceed the 4.5 ms limit. (B) Data points obtained in the 2 cases misclassified by the measure of latency interval

nificant to differentiate between cochlear and retrocochlear pathologies In cases of retrocochlear pathology we observed mainly normal waveforms but also broad and abnormal patterns and in the group of cochlear disorders these types were also observed together with recruiting and dissociated patterns

In pathological cases when the audiogram is normal or shows only a moderate loss the latency of P_{4-5} alone can serve to diagnose a retrocochlear disorder Typical results obtained in two cases one Meniere and one unilateral acoustic neuroma are presented on Fig 2 It appears clearly that in these particular cases on the basis of BER alone a retrocochlear lesion can be detected as it produces an abnormal increase in the absolute latency of P_{4-5} as well as a clear interaural latency difference Of course the latency interval between ECochG N_1 and BER P_{4-5} is also abnormally increased

In cases where the audiogram is noticeably altered as was the case for most of the patients in the cochlear and retrocochlear groups the latency of BER P_{4-5} is significantly modified which makes its interpretation difficult when considered alone Such an example is presented in Fig. 3 This patient did not show any sign of retrocochlear pathology but due to the high frequency loss the BER latencies present the same abnormalities as those reported above in the case of acoustic neuroma If the results of ECochG are taken into account no misinterpretation is possible since the N_1 latency also appears delayed and the time interval measured between ECochG N_1 and BER P_{4-5} can be identified as normal As can be seen on the traces the first peak of BER (P_1) is undetectable at any intensity and therefore the ECochG was essential for the interpretation of BER responses In cases where the audiogram presented a hearing loss of 30–40 dB or more the P_1 of BER could not be detected in the patients with a retrocochlear pathology whereas in the cochlear group most

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M Portmann
Clinique Universitaire d'O R L.
Laboratoire d'Audiologie Expérimentale
(F.R.A Inserm no 39)
Université Bordeaux II
Hôpital Pellegrin
Place Amélie-Raba-Léon
33076-Bordeaux Cedex
France

sented in Fig 7 No BER P_1 was detectable in recordings from the affected side so that with BER alone it might have been assumed that the peripheral organ was not functioning In these cases ECochG was also essential to diagnose the retrocochlear impairment

Finally comparison of the click thresholds for ECochG and BER might be another index for the diagnosis of retrocochlear pathology The threshold differences observed in all subjects in this study are reported in Fig 8 For the normal and cochlear groups threshold differences are clustered around 10–20 dB whereas in the retrocochlear group 6 among the 14 subjects presented a threshold difference between 20 and 50 dB Such threshold differences seem to be a valuable indicator of retrocochlear disorder but more data are needed to statistically validate its significance

CONCLUSION

The results presented in this study although limited to a rather small number of subjects clearly demonstrate the efficiency of combining both ECochG and BER recordings to diagnose retrocochlear disorders Both methods alone have serious limitations but together they offer a variety of criteria which give them a very powerful approach Some of these criteria are well established an absence of BER waves with a clear ECochG response an increased ECochG N_1–BER P_{4-5} latency interval and an ECochG threshold better than hearing threshold Several other possibilities particularly BER–ECochG threshold differences and interaural comparisons need further investigation

In cases of suspicion of retrocochlear disorder the information afforded by these electrophysiological methods is now considered as important as the results of other classical methods

ACKNOWLEDGMENT

This work was supported by grant INSERM no 79 1 418.6

RÉSUMÉ

Enregistrements transtympaniques et de surface dans le diagnostic des lésions rétrocochléaires

Tandis que l'électrocochléographie transtympanique (TT ECochG) permet l'étude détaillée de la fonction périphérique l'enregistrement en surface des réponses évoquées du tronc cérébral (RETC) permet l'évaluation de la conduction nerveuse jusqu au niveau du colliculus L ensemble de ces réponses a été simultanément étudié chez des sujets pour lesquels une tumeur cérébrale affectant les voies auditives a été confirmée chirurgicalement par la suite Des informations intéressantes ont pu être obtenues à partir de 1) la comparaison des amplitudes des composantes sensorielles et nerveuses dans les réponses ECochG 2) la comparaison des seuils ECochG, RETC et audiométriques 3) la mesure de l'intervalle de temps séparant la réponse du nerf auditif (pic N à l'ECochG) et celle du colliculus (pic P_{4-5} des RETC), 4) les comparaisons ipsi et contralatérales. Les mêmes mesures effectuées dans les cas de pathologie purement cochléaire principalement le syndrome de Ménière ont montré des différences significatives.

ZUSAMMENFASSUNG

Transtympanale und Oberflächenableitungen in der Diagnose retrocochlearer Storungen

Eine Anzahl von Patienten mit später operativ verifizierten intracerebralen zu einer Beeinträchtigung der Hörbahn fuhrenden Tumoren wurden mit Hilfe der transtympanalen Elektrocochleographie (ECochG) und durch Oberflächenableitungen von Brainstem-evoked Responses (BER) untersucht Wahrend die ECochG eine genaue Untersuchung der peripheren Funktionen ermöglicht, kann mit Hilfe der BER die Nervenleitung bis hinauf zum Colliculus Inferior gepruft werden. Dabei konnten wertvolle Informationen erhalten werden durch 1) einen Amplitudenvergleich von sensorischen und neuralen Komponenten des ECochG 2) einen Vergleich of ECochG BER und Horschwelle 3) Messungen des Zeitintervalls zwischen Hornervenantwort (ECochG-Komponente N) und der Antwort des Colliculus Inferior (BER Welle P_{4-5}) und 4) Seitenvergleiche Entsprechende Messungen bei pathologischen Cochleabefunden, hauptsachlich bei Ménièrescher Krankheit ergaben deutlich signifikante Unterschiede

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These filters were regulated for the following frequencies low pass 140 Hz, and high pass, 1 6 kHz. The amplifier gain was maximal i e 5 volts. This amplifier was connected by a cable to the head-rest in the incubator

(II) A modulator with a memory of 1024 words The analysis time was 20 ms It was equipped with an automatic artefact rejection system It was possible to effect any number of passages between 1 and 9900 In general for evoked potentials 2048 stimuli were used.

(III) A stimulation control module to determine the number of clicks per second In the cases observed the recurrence frequency was fixed at 20/s.

(IV) A trigger to synchronize analysis and sound stimulation.

(V) An oscilloscope

(VI) An X/Y recording table

(VII) An AX6 system for oscilloscope reading of recordings stored in the memory with the possibility of channel selection permitting inversion of the polarity of stimulus.

(VIII) A click generator (100 μs)

(IX) A TDH 39 earphone equipped with a flex.

The examination proper

The examination requires particularly stringent conditions of asepsis First the technical team was subject to the rules concerning entry into the Premature Unit that is removal of overalls and shoes and donning of sterile overalls and slippers A cap was also necessary Masks were not compulsory It was necessary to scrub up for 10 minutes.

The baby was brought into the examination room in its incubator All the connections remained constant so that the conditions of humidity and temperature did not vary The baby was brought in by the nurse in charge who remained present throughout the examination

The examination was timed to come after changing and feeding The babies slept throughout the examination and thus remained perfectly calm.

The electrodes used were of the electroencephalography needle type They were inserted intradermally The positive electrode was placed at the vertex The differential electrode was placed on the mastoid of the ear being tested and the earth on the forehead The impedance of these electrodes must not exceed 5000 Ω Faulty impedance due to incorrect positioning of the electrodes greatly hinders correct examination It leads to automatic artefact rejection according to the amplitude of the response The electrodes were placed by the nurse

The earphone was placed on the baby s ear In 17 cases the left ear was examined and in 2 cases the right ear

The parameters were then fixed the pass band of the amplifier between 140 and 1600 Hz, and the gain 5 μVolts Analysis time was fixed at 20 ms the recurrence interval at 20/s

RESULTS

The results are obviously incomplete They are interpreted with great caution because the series is so short.

Pattern

What is remarkable is that whatever the gestational age or the weight of the baby the pattern clearly shows Jewitt s five waves provided the high intensity recording 90 or 80 decibels, was observed

It is nevertheless important to note that if the baby was calm the J wave could be followed as far as 30 dB without any risk of error and in favourable conditions as far as 20 dB

In this first study amplitude was ignored although it may be mentioned that it decreased in proportion to the intensity of the stimulus

Latencies

Three intensities 80 60 and 50 dB S P L. were selected for the study of latencies The latency of the J wave and the difference between AP and J were measured more specifically

Since the examination was carried out in the

Acta Otolaryngol 89 370–375 1980

A STUDY OF BRAIN STEM EVOKED RESPONSES IN PREMATURES

A Morgon and B Salle

From the Centre d'Audiophonologie and the Prematu e Unit at the Hôpital Edouard Herriot Lyon France

At the Centre d'Audiophonologie an approach to auditory function in premature has previously been attempted in two studies (i) one which examined the reactions of premature to auditory stimulations (ii) the other examined the stapedial reflex in prematures

In the light of these studies it appeared that as audition matures it progresses from lowpitched to high pitched sounds and from sounds of greater to those of lesser intensity In other words the more premature the baby the greater the necessity to use intense low pitched sounds in order to evoke a response

The use of electrophysiological techniques with prematures involves major difficulties with vertex potentials and considerable difficulties when it is necessary to attempt electrocochleography

With the brain stem responses it is possible to examine auditory function in prematures provided certain essential conditions are fulfilled Prematures are extremely delicate and are particularly susceptible to infection

In addition to the physiological value of this examination there is a therapeutic consideration It is sometimes absolutely necessary to use an ototoxic drug in the treatment of a premature and its toxic effects on the cochlea can be monitored by means of BSRA

PROCEDURE

This study was performed in the recently opened Premature Unit of the Hôpital Edouard Herriot Lyon The Unit is part of the same hospital complex as the Centre d Audiophonologie In this Unit the prematures are maintained under computerized supervision. For examination purposes an electrophysiological unit has been installed

The difficulties involved in this type of experiment account for the fact that the number of cases is rather limited

The population

Fifteen cases representing 19 recordings were retained The pathological cases are excluded Two of them are quoted as an example in addition to these statistics None of the children involved in the study presented any pathological reaction of any kind

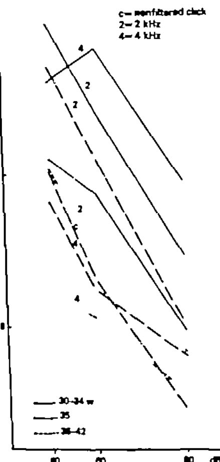
All the babies were in incubators They were not selected The recording team arrived on a specified day The Unit supervisor indicated a normal premature available for examination An effort was made to examine very prematures i e those with a low gestational age and low weight

Three of the 15 prematures were examined more than once 2 of them twice and one three times

The age range of these prematures varied from 30 to 42 weeks (g.a) The weight range was from 1 100 g to 2 050 g The time between admission to hospital and examination varied from 55 days to 1 day

Equipment

The equipment used for recording brain stem in prematures was MEDELEC manufactured by RACIA It consists of (I) A modulator–amplified and a pre-amplifier with filters



l Latencies *J* in function of gestational age

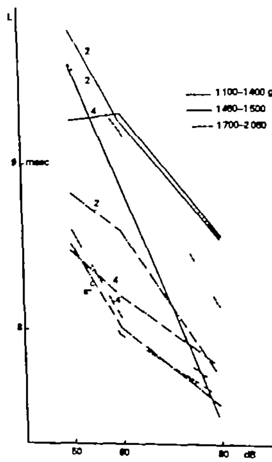


Fig 2 Latencies *J* in function of weight.

Respective influence of age and weight *J* ...ve latency for non-filtered clicks was com...red in the four conditions where the vari...les were as follows gestational age alone ...eight alone weight and age in two groups ...eight and age in three groups

Gestational age proved to be the most im...ortant factor but the influence of weight was ...reater for the lower weights and with lower ...tensity stimulation, that is 60 and better still ...0 dB

...ittle opportunity to observe ...athological recordings

...ur experience was limited to one case of a ...ull-term baby weighing 3300 g admitted to ...ospital with pyocyanic septicaemia treated ...with amikacin This baby died The B S R.A recording performed a week before death was completely flat

A single extended study in ideal conditions of one baby examined three times

1200 g, 35th week
1500 g 38th week
2050 g, 42nd week

The quality of the recordings made it possible to measure the latencied of J J_{III} J and J_{VI} waves. Latency at 2050 g reached at the 42nd week full-term, was not the same as for an adult but AP–J_V was close to it.

CRITIQUE

The major criticism of the present study is the limited number of observations made In fact

Table I

Name	Date of birth	Gest. age (weeks)	Weight (g)	Date of examin.	Gest age (weeks)	Weight (g)	Duration of hosp. and weight
Rémi B	09 04 79	33	1 390	?0 04 79	35	1 350	09.04–18 05/1 960
Hayat M	13 03 79	32	900	19 04 79	37	1 ?00	13 03–30.05/2 090
Cyril J	10 05 79	34	1 640	17 05 79	35	1 500	10 05–11.06/2 100
Laurence L. 1	?9 05 79	29	780	13 07 79	35	1 ?00	
Laurence L.	–	–	–	25 07 79	37	1 450	–
Sabine V	31 05 79	30	1 450	31 05 79	30	1 450	
Benjamin B 1	28 04 79	30	1 550	10 05 79	31	1 400	28 04–02.07/2 ?60
Benjamin B	–	–	–	31 05 79	34	1 500	–
Nelly N	20 01 79	30	1 000	23 02 79	35	1 300	20 0. 11 04/? 120
Chrystelle M	06.04 79	32	1 050	11 05 79	37	1 330	06.04–11.07/2 3?0
Adrienne C. 1	05 03 79	33	1 170	23 03 79	35	1 100	05.03–04 05/2 1?0
Adrienne C	–	–	–	1? 04 79	38	1 500	–
Adrienne C 3	–	–	–	03 05 79	4?	050	

absence of any pathological phenomenon the two criteria likely to have any influence were gestational age and weight

Influence of gestational age The recordings were divided into three groups (1) 30–34 weeks gestational age (2) 35 weeks (3) 36–42 weeks

The figures obtained were significant for both AP-*H* and *J* latency As hearing ma tured the latency of the *J* wave diminished It should be noted that the J_v wave for non filtered clicks and the J_v wave at 4 000 behaved in a similar fashion whereas latency remained high at 2 kHz

The decrease in AP-J_v was not linked ex clusively to the shortening of J_v latency but also to modification of the J_1 latency

Influence of weight The observations were divided into three groups according to weight 1 100–1 400 g 1 450–1 500 g 1 700–2 050 g

The results were not at all significant since those in the lowest weight category produced results which were almost identical with those of the group with the highest weight for non-filtered clicks at 4 kHz. Nevertheless the influence of weight can be ascertained

First the cases were divided into two groups (*a*) babies aged between 30 to 35 weeks and weighting less than 1 450 g and (*b*) babies aged between 36 to 4? weeks and weighing more than 1 450 g The results were significant only for non-filtered clicks and 4 kHz.

Second then the cases were divided into three groups (*a*) babies aged between 30 to 34 weeks and weighing less than 1 500 g (*b*) ba bies aged 35 weeks and weighing less than 1 500 g and (*c*) babies aged between 36 to 42 weeks and weighing more htan 1 450 g The re sults were then significant

Table II

Name	Date of birth	Gest age (weeks)	Weight (g)	Date of exam.	Gest age (weeks)	Weight (g)	Dur of hosp and weight
Pierre D	28 1 78	39	1 580	22.01 79	42	1 800	28 1 78–06.03 79/2 120
Ahmed N	05 03 79	32	1 890	18 03 79	34	1 800	05 03 79–03 04 79/? 160
Emilie N	19 02 79	32	1 820	26 0? 79	33	1 820	19 02.79–12 03 79/2 120
Angélique D	13 03 79	33	1 7?0	06.04 79	35	1 700	13 03 79–19 04 79/2 110
Véronique B	7 1 78	?7	1 100	01 02 79	39	1 700	08 1..78–19 0? 79/1 750
Alexis D	14 01 79	35	2 020	23 01 79	36	1 700	07 12 79–28 01 79/2 220
Adrienne C	05 03 79	33	1 170	03 05 79	42	050	05 03 79–04 05 79/? 1?0

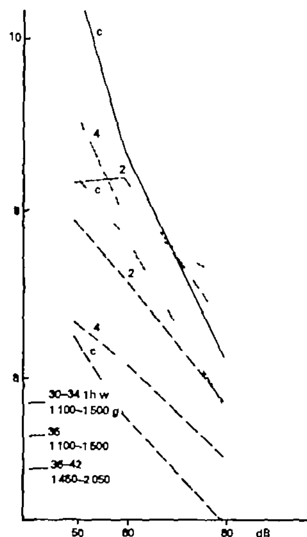
DISCUSSION

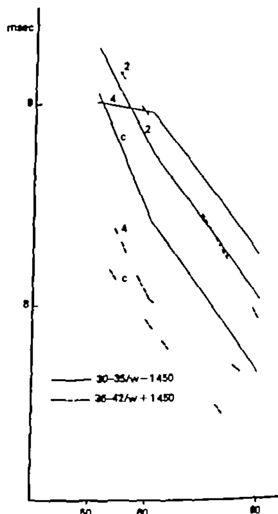
Sclala to Morgon. Did you use in your studies any frequencies other than 2000 Hz and 4000 Hz

I our studies on 4 premature babies and 141 full-term neonates the best responses on application of ERA ere obtained by using 900 Hz in prematures and 1000 Hz in full term neonates. There were only single responses on stimulating ith 000 Hz. What is your comment on this?

Morgon (Reply).

The choice of frequencies depends on tw factors: (i) to keep the same procedure in baby infant or adult, and (ii) t use frequencies to cover the larger part of the frequencyfield.





Figs 3–4 Latencies *J* in function of weight and gestational age

no measurement was made unless five results were obtained for a given intensity and frequency

The work continues It is the subject of a thesis which will collate more informations and a greater number of observations either conforming or contradicting these first results

Nevertheless in spite of the procedural difficulties the observations presented here were obtained in ideal conditions providing easy reading of the recordings

CONCLUSIONS

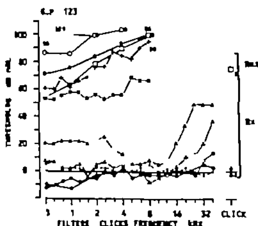
The study of B S R A in premature in cubators reveals the following four points

Auditory function matures as follows non filtered clicks and the 4 000 Hz frequency develop in a parallel fashion The 2 000 frequency seems to be delayed

The latency figures for the J_V wave dim ish as hearing matures but the premat baby does not have the same latencies at t term as the new-born baby AP–J_V also minishes as maturity nears principally b cause of the reduction in J_V latency but a to a certain extent because of the modific tions in J_I latency wich oddly enough seems lengthen

The most important element remains ges tional age but the influence of weight cann be totally eliminated

B S R.A appears to be a good method b which to survey premature babies in the nor of the incubator or/and with ototoxic trea ment



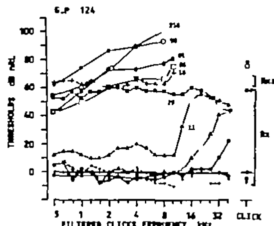


Fig. 1. Frequency threshold curves of the responses recorded at the round window for guinea pigs at various times indicated in days (*small characters*) after the beginning of the first amikacin treatment (day 0). Note the rapid evolution during the first treatment (Rx1: 14 × 450 mg/kg/day) and the small changes during and after the second treatment (Rx2: 20 doses), particularly for GP 124 (*right*).

while the other ear, not involved by the procedure, served as a control at time of sacrifice of the animal. The surgical procedure as well as the method of stimulating and recording from this electrode in awake animals have been described in detail elswhere (Aran & Erre, 1979) and used in various studies (Aran & Darrouzet, 1975; Aran & Cazals, 1978). As usual, the auditory nerve compound action potential (CAP) thresholds to filtered clicks from 0.5 to 40 kHz are determined (CAP audiograms), the intensity levels being referred to the mean threshold of normal responses (dB nRL). Although we normally use a Bruel & Kjær condenser microphone cartridge (4134) directly coupled to the ear as a transducer, in these experiments as soon as the thresholds became elevated we had to use a TDH 39 Telephonics earphone in order to obtain higher sound levels, although the frequency of the transient stimuli could not then exceed 10 kHz. Responses to the unfiltered click using this earphone were also recorded from the very beginning of the experiments so that patterns of the responses and input–output amplitude and latency functions could be monitored over the entire experiment. As usual for the recordings we systematically used averaging and clicks of alternate polarity to cancel out any microphonic potential.

Effects of the first treatment

These 5 implanted guinea pigs, having normal click-evoked responses and CAP audiograms, received a first treatment of amikacin identical with that used in our earlier studies (Aran et al., 1979; Cazals et al., 1979*b*), i.e. 14 intramuscular injections of amikacin (daily except during week-ends) at doses of 450 mg/kg. Responses were monitored every 2 to 4 days before and also during the treatment, then with longer time intervals between tests after them.

For all the guinea pigs, CAP audiograms started to alter already at the end of the first week of treatment, beginning with an elevation of the high frequency thresholds (Fig. 1). By the end of the treatments, all the CAP audiograms showed the same pattern with thresholds around 50–70 dB at 0.5 kHz and progressively increasing with the frequency up to 90–100 dB at 8 kHz.

Then shortly before or after the end of the treatment, 2 guinea pigs died (at 14 and 20 days).

By this time the click-evoked responses were profoundly altered in all the animals

Acta Otolaryngol 89 376–383 1980

ELECTROPHYSIOLOGICAL MONITORING OF THE COCHLEA DURING AND AFTER TOTAL DESTRUCTION OF THE ORGAN OF CORTI

Jean Marie Aran Yves Cazals Renaud Charlet de Sauvage
Anne Guilhaume and Jean Paul Erre

From Laboratoire d'Audiologie Expérimentale (F R A Inserm No 39) Clinique Universitaire d'O.R.L. (Dir Pr M Portmann) Bordeaux France

Abstract A series of acoustically evoked potentials can be recorded from the cochlea up to the auditory cortex in guinea pigs where the organ of Corti has been totally de stroyed after extensive treatment with amikacin, but where some of the spiral ganglion neurons always remain and where the vestibular receptors are only slightly affec ted The cochlear responses have been monitored in guinea pigs permanently implanted with a round window electrode and receiving such treatment The normal audi tory nerve compound action potential disappears within a few days, while the very typical response (diphasic short latency (0 3 ms) small amplitude) appears This response then remains remarkably constant in time as far as we could observe (up to almost one year) This response might be a component of the normal response undiscern ible under normal conditions but revealed by the selec tive impairment of the labyrinth by amikacin in contrast to the global effect of every other known otodestructive agent Some basic questions still remain which fibres are effectively stimulated (cochlear or vestibular) what are their central projections and what kind of sensa tion is conceivably associated with these responses?

Clear click-evoked electric responses have been recorded at the round window of guinea pigs cochleas which about one month after in tense treatment with the aminoglycoside anti biotic amikacin presented a total loss of inner and outer hair cells along the entire length of the basilar membrane except for a very few remaining outer hair cells in the third row at the extreme apex while the vestibular func tion and receptors seemed to be preserved and some nerve fibres survived in the remnants of organ of Corti (Aran et al 1979)

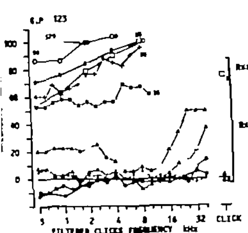
In subsequent experiments identical results have been obtained in the same conditions and the effects of phase of the stimulus anoxia and masking on such responses have strongly suggested that they were of a neural nature (Cazals et al 1979*b*) These responses are followed by an activation of the central nervous system and of the auditory cortex as evidenced in recordings from surface electro des and from electrodes on the auditory cor tex However the brainstem recordings show an absence of waves IV–V complex and cor tical responses although spescifically limited to the auditory cortex are delayed by about 4 ms as compared with the click-evoked re sponses in the normal guinea pig (19 instead of 15 ms) (Cazals et al 1979*a*) (see an example in (Fig 5)

In order to obtain more information about the nature of the early cochlear responses, their onset and evolution in time during and after (up to one year) the same—or more in tense—amikacin treatment have been studied either together or separately on the same or different guinea pigs For this purpose we used the method of chronic recordings of cochlear responses in awake animals complemented by acute recordings from both ears immediately before sacrifice We report here the results of various experiments in which unless other wise specified the times of observation are re ferred always to the first day of treatment for each animal (day 0)

Short term Evolution during Amikacin Treatments

Methods

Five normal pigmented guinea pigs (GP) were equipped with an electrode permanently im planted on the round window of the left ear



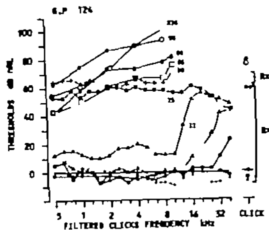


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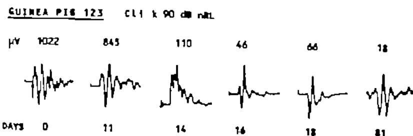


Fig 2 Patterns of averaged click-evoked responses at the round window (using clicks of alternate polarity to cancel out any microphonic potential) at various times for GP 123 The numbers above the traces indicate the peak-to-peak amplitude in microvolts of the corresponding response The last trace on the right (day 81) is that recorded at the end of the 2nd treatment (see Fig. 1) Trace duration 5 ms, click at the tympanic membrane 0 9 ms after the beginning of the traces (*arrow*) Note the important positive component which disappears from day 16 to day 18

showing the typical characteristics already observed in the earlier experiments about one month post Rx i e high thresholds (50 to 70 dB nRL) very short latency (0 3 ms) and amplitude of the order of 100 μV at 100 dB which although 10 times smaller than in the normal gives very clearly discernible responses even without the use of averaging (Figs 2 and 3) However for one guinea pig (GP 124) the responses were less affected and these characteristics were established only during a second more extensive treatment (Fig 3)

Effects of a second longer treatment

In the 3 remaining guinea pigs (GPs 117 123 and 124) a second treatment using the same daily dose was administered but over 20 injections (4 weeks) instead of 14 starting at day 56 (that is 38 days after the end of the first treatment) The responses were similarly monitored in 2 guinea pigs (GPs 123 and 124) Electrode failure prevented such monitoring in the third one (GP 117) though it could be used for the long term study by testing the right ear at time of sacrifice (at day 216) as revealed later

During this second treatment there was a slight elevation of the thresholds (Fig 1) but the waveforms were not notably altered for GP 123 while they progressively reached the typical pattern for GP 124 with the complete disappearance of an early positive component (summating potential?) clearly observed usually during the treatment (Figs 2 and 3)

Long term Evolution

By this time more than one month after the end of the first treatment—and particularly after the second one—we might say in view of the histological results in the earlier experiments (Aran et al 1979 Cazals et al 1979*b*) that the organs of Corti had been totally destroyed The constancy in time of such paradoxical responses acoustically evoked without organ of Corti have been further evaluated in guinea pigs from two different experiments in the 3 guinea pigs mentioned above (GPs 117 123 and 124) and in 3 guinea pigs from our earlier experiments (GPs 189 195 and 196) (Cazals et al 1979*b*) which were not initially implanted and which had received only one series of 14 injections Responses were monitored in one of them (GP 189) implanted about 2 months after the end of the treatment (at day 85) until day 347 while the 2 other not implanted were sacrificed at day 262 just after acute recordings

GP 124 showed remarkably constant responses until the sacrifice more than 7 months later (at day 216) (Figs 1 and 3) However for GP 123 the progressive threshold elevation continued after the end of the second treatment until no response was recordable at 4½ months (Fig 1) At time of sacrifice (>7 months) responses could be recorded from

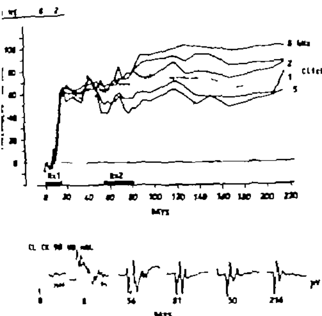


Fig. 3. *Upper curves.* Evolution of the thresholds to the various filtered clicks (from 0.5 to 8 kHz) and to the click ( ) during the entire experiment including the amikacin treatments (Rx1 and Rx2). Note the relatively good low frequency thresholds. *Lower trace.* Click-evoked responses from the same guinea pig at various times. Traces as in Fig. 2. Peak-to-peak amplitudes indicated in microvolts in small characters. Note the disappearance of the early positive peaks during the second treatment (*days 56 to 81*). *It is not obvious whether the final response (at days 150 or 216) is present or not as the responses at 0, 18 or 56 days.*

the ears and from the auditory cortices (with contralateral acoustic stimulation) in GPs 124 and 117 (Figs. 4 and 5), while no response *was recordable either from ears or from the* auditory cortices of GP 123.

In GP 189, responses were remarkably constant both in pattern and amplitude from day 85 (implantation) to day 347 (sacrifice) (Fig. 6). Similar responses could be recorded in GP 196 (Fig. 7), while no response could be recorded from either the left and right ear of GP 195. However, for this GP it was obvious either *during electrode implantation for acute recordings* or later when preparing the ears for histology, that it had been suffering from bilateral otitis media (thickening of bone and presence of membranous material in the middle ears).

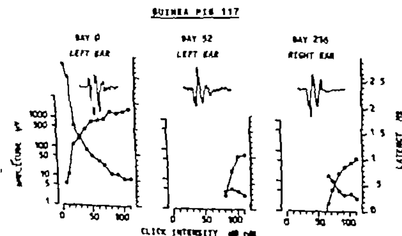


Fig. 4. Patterns of the click evoked responses at 90 dB nRL (*upper trace*) and input-output amplitude and latency functions at days 0 and 52 from the left ear, and at day 216 from the right ear of GP 117 which received the same treatment (Fig. 3) as GPs 123 and 124. Traces as in Fig. 2.

GUINEA PIG 124

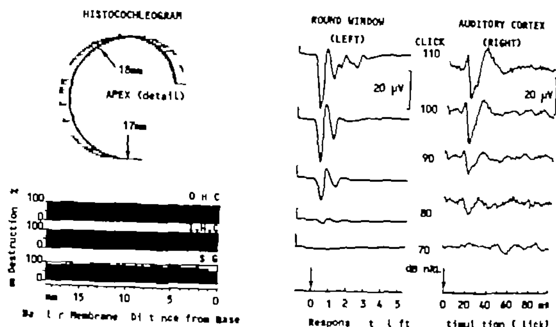


Fig 5 Histocochleogram of the left ear and responses to left ear stimulation from the round window of the left ear and from the right auditory cortex recorded the day of sacrifice (day 216) for GP 124 (see *Figs 1* and *3*) Note the only remaining hair cells (●) displayed in the detailed reconstruction of the apex (each 10 µm section has been inspected and represented) Arrows with numbered millimetres indicate distance from the oval window Note the total inner hair cell (I H C) loss the almost total outer hair cell (O H C.) loss and the small proportion of remaining spiral ganglion neurons (S G) along the basilar membrane Note the good correlation between round window and auditory cortex responses Arrival of the click at the ear time 0 (*arrow*)

Histology

As in the former studies again no hair cells inner or outer could be detected along the entire length of the basilar membrane in any of the guinea pigs except for very few outer hair cells of the third row at the extreme apex In several guinea pigs we used a serial section technique and by sectioning the entire cochlea in 10 µm steps each 10 µm section was inspected so that no hair cell could be missed

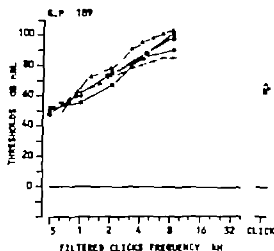


Fig 6 Frequency threshold curves and responses to the click from day 85 to day 347 fo GP 189 which received only one treatment of amikacin (14×450 mg/kg/day) Traces as in Figs 1 and 2. Numbers in small characters indicate the peak-to-peak amplitude of the responses in microvolts.

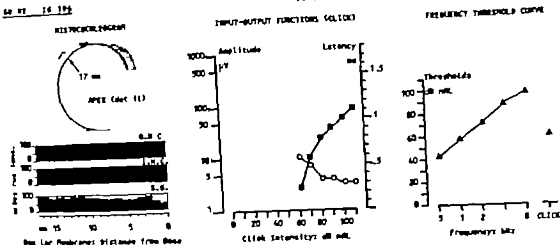


Fig. 7. Histocochleogram (as in Fig. 5), of GP 196 which received the same single treatment as GP 189 but was sacrificed at day 262 instead of 347, and input-output amplitude and latency functions (click stimulation) and frequency threshold curve of the responses recorded from the round window the same day. This guinea pig typifies the cochlear condition and types of response obtained after such treatment.

Then no more than a dozen outer hair cells could be counted. Examples are shown in Figs. 5 and 7. When counting the spiral ganglion neurons as well, there appeared to be a significant loss, more pronounced at the apex than at the base, and also more pronounced after the two treatments than after only one for the same delay between treatment and sacrifice (>7 months). Also the loss seems more serious after 7 months than after 2, indicating a slow, progressive neural degeneration. However, in any case some spiral ganglion neurons still remain and, as seen in electron microscopy, a few nerve fibres running in the epithelial layer which covers the basilar membrane to varying extents. Also the vestibular receptors always appear much less affected. The condition of the ears of GPs 1.3 and 195, where no response could be recorded, is comparable to the others.

DISCUSSION

Two main items of information about these paradoxical peripheral responses, acoustically evoked in the absence of the organ of Corti, are given by the short and long-term studies respectively.

These responses appear as soon as the normal compound action potential disappears. For all the guinea pigs this occurs within a very short transient period (threshold shift of about 60 dB within one week). At this time it is indeed difficult to understand the recorded patterns which obviously contain a relatively large proportion of positive d.c. components like the summating potential, disappearing progressively. Then the typical response such as that observed in our earlier experiments at about 1–? months after the end of the treatment is clearly established. Although this response would not be discernible in the normal response before treatment, due to the too large amplitude ratio, it might be possible to detect it in the distorted, smaller patterns during the transient period (Figs. 2 and 3).

The response characteristics are impressively stable over several months (Fig. 6). In contrast, during one earlier study over 7 months, using kanamycin with similar daily doses but a shorter treatment (8 doses), a close parallel between progressive hair cell loss (as supported by many histological studies, partic

ularly that of Gonzalez et al 1972) and a progressive change of the responses could be demonstrated In the present condition using amikacin more extensively (14 doses) the hair cells disappear completely and quickly as early as one montth post treatment (Aran et al 1979 Cazals et al 1979*b*) possibly earlier There is certainly a progressive spiral ganglion degeneration which however is difficult to correlate with the evolution of the responses Admittedly there are 2 guinea pigs (GPs 123 and 195) without responses but for one (GP 195) it could be ascertained that there was an added conduction loss For GP 123 we do not have any such evidence but it is the only one in our series of experiments which could infirm the other data although a conductive impairment in this case cannot be ruled out

It must be recalled that these responses seem to be effective for the activation of the central nervous system and particularly the auditory cortex In effect whenever the auditory cortex responses were investigated in these and other experiments the results always correlated very well (thresholds) with that obtained at the periphery (Cazals et al 1979*a*)

These two observations rapid onset and long term stability indicate that the peripheral responses might originate from an already existing system in contrast to the slow neuromorphological changes which occur after such drastic impairment of the cochlear structures (Spoendlin 1975) It is likely that this system lies in the vestibular receptors such as the saccule which as already mentioned is only slightly affected However the vestibular receptors are also preserved during other treatments (e g kanamycin) Moreover on the basis of the present data it cannot be assessed whether the structure responsible for these responses is already functioning in the normal or does so only in such a clear-cut pathological condition

Thus there still remain some basic questions some of which should be answered soon (i) Which nerve fibres are effectively stimulated and responsible for these responses (cochlear or vestibular fibres)? (ii) What are their exact central projections? (iii) finally what kind of sensation if any is evoked in such conditions?

ACKNOWLEDGEMENT

This work was supported by INSERM grant no. 8-ASR4 (Oreille interne)

RÉSUMÉ

Des réponses évoquées par des stimulations acoustiques peuvent être enregistrées, depuis la cochlée jusqu'au cortex auditif chez des cobayes dont les organes de Corti ont été entièrement détruits lors de traitements intenses par l'Amikacine mais où subsistent des neurones du ganglion spiral et où les récepteurs vestibulaires semblent peu affectés Les réponses cochléaires ont été suivies chez des cobayes implantés d'une électrode à demeure sur la fenêtre ronde et recevant de tels traitements. Le potentiel d'action composite normal du nerf auditif disparaît en quelques jours alors qu'apparaît cette réponse caractéristique (diphasique de latence courte (0,3 ms), de faible amplitude) Cette réponse demeure alors remarquablement constante dans le temps aussi longtemps que nous avons pu observer (preque un an) Cette réponse pourrait être un élément de la réponse normale masqué par celle-ci dans le conditions normales, mais démasqué lors de l'atteinte sélective du labyrinthe due au traitement d'Amikacine par opposition à l'action globale de tout autre agent otodestructeur connu Cependant quelques questions élémentaires sont toujours posées, quelles sont les fibres effectivement stimulées (cochléaires ou vestibulaires) quelles en sont les projections centrales et quelle sorte de sensation pourrait être associée à ces réponses

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J-M Aran
Laboratoire d'Audiologie Expérimentale
(E R.A Inserm no 39)
Université Bordeaux II
Hôpital Pellegrin
Place Amélie-Raba-Léon
33076-Bordeaux Cedex
France

DISCUSSION

Spoendlin to Aran In some of your animals you recorded acoustic potentials in spite of an apparently complete loss of hair cells and spiral ganglion cells. This is in fact very surprising and can only be explained by an acoustic activation of some unaffected vestibular elements I would also question any correlation between the ECOG response and functioning cochlear neurons. The quantitative morphological evaluation of the cochlea is of course crucial for the interpretation of these results Such quantitative critical evaluation is probably very difficult on the basis of serial sections and I think combined surface and EM technique would be better In our experience it can easily happen that one finds distorted rudimentary hair cells in EM preparations of cochleas where in LM all hair cells seem to have disappeared

Burian to Aran It is very difficult to find an explanation for the described observation If we assume that all hair cells are destroyed and not merely damaged and still functioning, we have to think of vestibular transformation. This vestibular source of the potentials seems to be very improbable—otherwise we would find some potentials more frequently in completely deaf patients. One hypothetical explanation seems to be a sinister phenomenon we all know named electrophony As it is based on electromechanical transformation it could also be possible that mechanoelectrical transformation produces an electrical stimulation of the nerve fibres.

Gibson to Aran. I ask whether any adaptation studies have been performed This would distinguish between a receptor potential or an artifactual microphonic and a truly neural component.

Brackley to Aran. This response should perhaps be called hair-cell less acoustic response rather than a cortiless response Presumably the other cells of the organ of Corti are present?

BM Johnstone has postulated direct mechanical-stimulatory effect on the nerve fibres supplying the IHCs. It is possible that this is the origin of the response in the auditory nerve that Aran has described.

As part of the response has a short latency 0.3 ms, it suggests that some form of receptor potential is involved. It would be interesting to do d c. recordings of the scala media to see if there is an endolymphatic d.c potential present in the ears damaged with Amikacin

Baxer to Aran It would be interesting to know the frequency spectrum of the click stimulus Assuming that it is response of the saccule, strong click may cause sudden dislocation of the stapes which might be a proper stimulus for the saccule If this is so have you observed any movements of the eyes by nystagmography?

Aran (Portmann repl)

Thank you for your comment I the absence of Dr Aran I will try to do my best and invite you to our laboratory to see exactly what Dr Aran and his group do

They carefully observed with light and electron-microscopy and they believe that the hair cells are destroyed.

The behaviour of the response proves that it is not an artefact. I thank the speakers for the ideas they gave to try to explain the phenomenon

Acta Otolaryngol 89 384–387 1980

CLINICAL AND EXPERIMENTAL STUDIES ON THE INCORPORATION OF MATERIALS APPLIED AS COVERING FOR THE LABYRINTH WINDOW

O Ribari J Jori and I Tomity

University ENT Clinic Szeged Hungary

Abstract From among the materials used for covering the labyrinth window of the foreign materials gelfoam proved ideal from clinical and experimental points of view while of the materials of interstitial origin fascia, perichondrium and periosteum seem to be suitable for covering the labyrinth window resp for transplantations in the tympanic cavity

Otosclerosis is one of the forms of conductive hypacusia that can be best corrected In 1956 Shea introduced stapedectomy since when hundreds of thousands of otosclerotics have regained their hearing thanks to surgical operation

Otologists use various materials for stapedectomy Some operating otologists do not use foreign material in the tympanic cavity In this way for instance Portmann uses the stalk of the stapes to replace the removed stapes Bauer forms a cortical columella replacing the removed stalks with that

Some operating ear sugeons use plastic teflon prostheses for replacing the stalk of stapes Most of them—Schuknecht (1963) House (1970) and also we ourselves (Ribari 1976)—apply rustproof metal This rustproof metal may be steel tantalum or molybdenum as well For covering the oval window or the round window after rupture various materials are used for instance gelfoam perichondrium periosteum vein fascia fat and possibly blood (Goodhill 1971)

The results of operations carried out with different techniques generally bring about a 90% lasting result

At the Oto-Rhino-Laryngological Department of the University Medical School in Szeged we have carried out comparative investigations partly on the clinical patient material and partly by animal experiments to see how the result of the operation is affected by covering the labyrinth window with different materials and how the implants are incorporated into the middle ear

In our operations at first venous wall was used with polyethylene tube which gave rise to some problems fistulization and development of granulation in the vicinity of the venous wall More recently we have covered the labyrinth window with gelfoam or else used temporalis fascia in some cases

Of late we have almost always applied a wire prosthesis in our operations In the case of an obliterative juvenile otosclerosis a piston was inserted In case of applying a vein, the closure of the post-operative airbone gap follows relatively slow The closure is often not even full

In Fig 1 a picture of a venous wall is shown removed from the oval window in connection with a reoperation The venous structure can be identified but a very strong rebuilding—a considerable fibrosis—can be observed too

The operation was performed 18 years ago The ossification of the removed vena can be observed in the interpositum The implanted veins were to be removed in more than one case It was proved by the operations that the venous wall formed a comparatively thick interstitial material the vibration of the thick

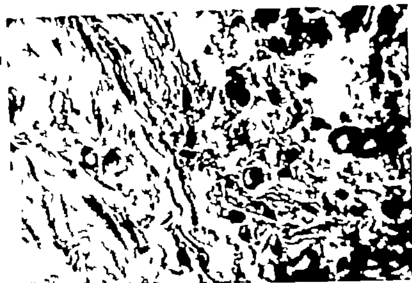


Fig 1



Fig 2

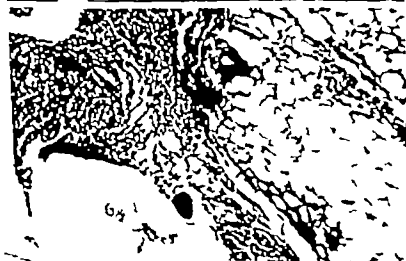


Fig 3

material was not satisfactory and at the same time a perforation could also develop on the implanted venous wall The insertion ad hesion of the vein was not satisfactory

We have carried out comparative investiga tions on 20 patients in our clinical patient material on one side the oval window was covered with gelfoam on the other side fascia was applied to cover the oval window one year later It was found by postoperative audiometry that in the case of a gelfoam–wire prosthesis the air–bone gap closes much faster than in cases where fascia alone is applied

One year after the operation was performed no gelfoam was found in the patient material nor any difference in the fasciae Thus we see that the late results were not affected by the difference between the two implanted materials

When applying gelfoam we have not experi enced a single case of toxic damage to the inner ear Gelfoam in our experience never behaves like foreign matter

Although we know that the conclusions drawn from animal experiments are not valid in every case in respect of man we have in vestigated in experiments made on guinea pigs how the incorporation of the materials used for covering the labyrinth windows takes place

At first we carried out the operation on guinea pigs under urethane anaesthesia by freeing the bony wall of the bulla ossea and then after removing the stapes covering the oval window with different materials gelfoam fascia perichondrium periosteum resp fat and closure of the wound

Of late we have already performed the operation via the auditory canal after deflect ing the tympanic membrane The animals were decapitated at different times one 4 6 weeks resp 2 months after the operation and the cochleae were removed together with the materials put on the labyrinth windows

It can be seen even by macroscopic inspec tion that gelfoam periosteum and other con nective tissue materials soon become incorpo rated in a majority of cases It can be seen in the figure that the incorporation of blood vessels begins very soon as well (Fig 7) Only in the case of fat transplantation have we observed frequent necrotizing of the fatty tissue and the development of otitis in animals. This did not occur (or only very rarely) when other materials were applied In most cases, the implant and even the fatty tissue incorpo rated very well

The material removed was fixed in formalin, decalcified stained and histologically exa mined The histological investigations also showed that the histological materials used were incorporated into the middle ear very well

Gelfoam shows an histological transfor mation already a week after implantation and the fascia also incorporates well one week after the operation In the place of gelfoam, connective tissue develops within a fortnight and the gradual absorption of gelfoam is vi sible The periosteum very soon adheres to the environment of the bone filling the window with a fine membrane The response of fatty tissue can be observed in Fig 3

At the edge of the fatty tissue granular tis sue develops 6 weeks after the operation pres sing forward somewhat towards the inner ear The same is discernible still better when mag nified strongly From the clinical results pre sented and the other histological investiga tions as well we have drawn the conclusion that as opposed to the histological interposi tion gelfoam is well suited for application to close the oval window The acoustic results in the case of applying gelfoam are also best in the early period

The advantage of gelfoam is that it is easi ly handled is plastic and closes the window very well The difference ceases to exist ac cording to the clinical data after the incor poration of the tissue Of the interstitial mate rials periosteum and fascia can be applied very well to cover the oval window On the other hand the venous wall and fatty tis

me proved to be a less good interpositum The venous wall partly calcifies is transformed and becomes stiff A part of the fatty tissue necrotizes another part forms in some of the cases, undesirable granular tissue

Before transplanting the materials we had attempted application of a low-energy helium-neon laser in order to observe how the irradiation of the low-energy laser affects the adhesion of transplantation Unexpectedly the low-energy laser promotes the vascularization and adhesion of transplantation considerably As a result of irradiation the transplant incorporates faster and vascularization also follows earlier

We have also applied irradiation to close the perforation The low-energy laser induced rapid epithelialization of the perforation.

We have applied irradiation to close experimental tympanic membrane perforations as well The low-energy laser induced rapid epithelialization of the perforation It was shown by the histological investigation carried out after the low-energy laser irradiation that no change arose On the other hand oedema was formed at elevated energy levels

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Prof Dr O Ribari
E.N.T Clinic
University Medical School
H-6725 Szeged
Hungary

DISCUSSION

Portmann to Ribári. I think that we have to avoid gelfoam for closure of the oval window I 1964 I did 300 cases with gelfoam and the sensorineural hearing loss went up from 0 7% to mor than 6%

I think that w must recommend absolute avoidance of gelfoam—if it is not toxic by itself which is not proved it does result in thin membrane which does not protect the labyrinth sufficiently against inflammation from pharynx and tubal region.

Kelemen to Ribári. Tissues acquire independence in late lif as in embryonic periods.

Ribári (Reply)

Thank you very much for your questions

The gelfoam made very thin membrane but it has no toxic effect.

It depends on the method of sterilization

An ideal material is fascia and perichondrium. Fat and vein are no good

Acta Otolaryngol 89 388–392 1980

DEVELOPMENT OF ACETYLCHOLINESTERASE (AChE) STAINING IN HUMAN FETAL AUDITORY CORTEX

J Krmpotić Nemanić I Kostović Z. Kelović and Đ Nemanić

From Department of Anatomy Drăgo Perović Medical Faculty Zagreb and ORL Clinic General Hospital Dr Mladen Stojanović Zagreb Yugoslavia

Abstract In this study acetylcholinesterase (AChE) staining methods have been used to demonstrate the development of the prospective afferent fibres of auditory cortex in human fetuses ranging between 8 and 28 weeks of gestation Earliest AChE positive staining was found in fetuses at 20–24 weeks in the neuropil of the marginal zone and throughout subplate layer of the auditory cortex On the basis of this characteristic AChE staining pattern the auditory cortex may be delineated from surrounding cortical areas At 24–26 weeks during intensive lamination of the cortical plate AchE-positive reaction appears in the deep part of the cortical plate In the oldest fetuses (28 weeks) a columnar pattern of vertical darkly stained zones was seen in the middle third of the cortical plate We conclude that AChE staining is characteristically distributed within cytoarchitectonic compartments and synaptic layers of the developing auditory cortex The AChE-positive layers coincide with the laminar pattern of synaptogenesis Thus AChE positive reaction during development may serve as a parameter of cortical afferent (thalamic?) innervation of the auditory cortex In addition AChE reactivity may indicate the specific transmitter nature of the afferent fibres in the auditory cortex

Our previous morphological observations (Krmpotić Nemanić Kostović Nemanić & Kelović 1979) suggested that circuitry elements (dendrites axons synapses) are present in the human auditory cortex in an early phase of prenatal development and have consistent spatial relationship to the cytoarchitectonic layers A similar pattern was also demonstrated on other sensory cortices in man and primates (Molliver Kostović & Van der Loos 1973 Kostović & Molliver 1974 Purpura 1975 Kostović Knežević Kostović Krmpotić Nemanić Kelović & Vuković 1978) In the light of the possible functional status of the early sensory circuitry in the human fetus (Vaughan 1975) it is necessary to determine afferent input to the developing cortical layers

The most promising morphological approach to the analysis of afferent cortical input in the human fetal cortex is to demonstrate ingrowing fibres by histochemical techniques (Kostović Kelović Krmpotić Nemanić & Kračun 1979 Kostović 1979) and to determine their prospective transmitter properties In the present study histochemical methods have been used to determine the spatial and temporal pattern of the development of the acetylcholinesterase (AChE) positive staining in the auditory cortex of the human fetus The AChE reaction is used primarily for demonstration of fibre ingrowth (Kostović Knežević Kostović Krmpotić Nemanić & Kelović 1979) and the pattern of cortical organization (Kostović 1979) In addition we plan to obtain some indicators of the prospective cholinergic innervation of the auditory cortex

MATERIAL AND METHODS

Brains of human fetuses ranging between 30–270 mm crown–rump length (8–28 weeks of gestation) were fixed for 48 hours by immersion in 0 1 M sodium phosphate buffer containing 1 5% glutaraldehyde plus 2% paraformaldehyde For histochemical analysis the blocks containing temporal cortex were serially sectioned on a freezing microtome Free sections were stained for AChE reaction with Lewis modification of Koelle s method (Km



Fig 1 Low-power micrograph of the superior temporal corte AChE staining Human fetus, 22 weeks Note boundaries of the prospective auditory cortex (*double rrow*) and approaching thalamocortical fibres (*arrow*). *CP* = cortical plate *SP* = subplate layer *P* = putamen *CI* = capsula interna, *CE* = capsula externa.

jević & Silver 1966) and Karnovsky Roots method (Broderson Westrum & Sutton 1974) The specificity of AChE reaction (specific or non-specific AChE) was controlled by various inhibition tests For cytoarchitectonic analysis adjacent sections were stained by the Nissl method In addition we have used serially sectioned celloidin-embedded brains from the neuroembryological collection of the Department of Anatomy Medical Faculty Zagreb

RESULTS

In the youngest fetuses (8–20 weeks of gestation) the developing cortical layers (marginal zone cortical plate and subplate layer—for definition of the cytoarchitectonic pattern see Krmpotić Nemanić, Kostović Nemanić & Kelović 1979) were free of AChE staining However in the underlying intermediate zone the AChE positive fibres were seen within capsula interna and capsula externa as early as 1 weeks of gestation In the next period (20–4 weeks) during intensive lamination of the cortical plate AChE staining of the neuropil was observed in two strata of the temporal cortex marginal zone and subplate layer (Figs 1 and) In the deepest part of the cortical plate we have seen only diffuse AChE staining (Fig. 2 asterisk) On the basis of the strong AChE staining in the subplate layer the auditory cortex may be delineated from the surrounding temporal cortex which shows very weak AChE reaction (Figs 1 and ?) The AChE-positive subplate layer is in continuation with the underlying AChE-positive fibre system of the capsula interna and fibre bundles which penetrate the striatal anlage (Fig. 1)

In fetuses of 24–26 weeks of gestation the AChE-positive reaction appears in the middle third of the cortical plate (Fig. 4 asterisk) This intensive AChE staining appears to be

Fig 2 AChE staining in the subplate layer and marginal zone (*MZ*) in the auditory cortex of 22-week human fetus (*arrows*). In the cortical plate weak AChE staining (asterisk) Bar 700 μm marks magnification for Figs. 7

Fig 3 Nissl-stained section (adjacent) from specimen show in Fig. 2.

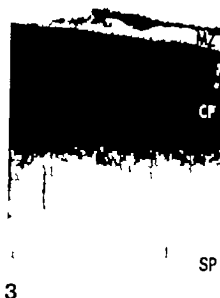
Fig 4 Auditory cortex. Human fetus, 25 weeks, AChE staining Note increased AChE staining in the cortical plate (asterisk)

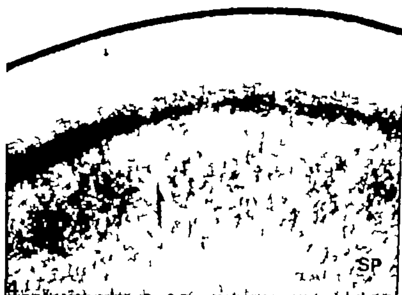
Fig 5 Nissl-stained adjacent section.

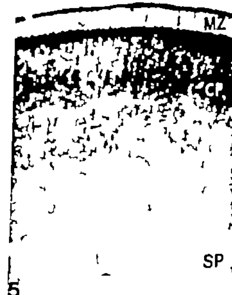
Fig 6 Darkly stained AChE-positive alternating (*arrows*) zones (*C* olumns) in the prospective layer IV of auditory cortex of the 28-week human fetus

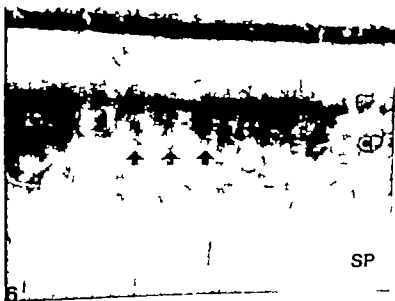
Fig 7 Nissl-stained (adjacent) section. Human fetus 28 weeks.


MZ
CP
SP
2


MZ
CP
SP
3


SP
4


MZ
CP
SP
5


CP
SP
6


MZ
CP
SP
7

ertically uniform There was a decrease in ie density of AChE staining within the sub- late layer (Fig. 4)

In oldest fetuses examined (28 weeks of estation) a pattern of vertical darkly stained lternating zones (140–200 μm wide) appeared within the AChE-positive middle third of the cortical plate (Fig. 6 arrows) These vertical positive zones (columns) are separated by nar rower interspaces of lower staining density AchE-positive columnar zones could be fol lowed in serial sections in a rostrocaudal direc tion

DISCUSSION

The observation presented in this paper pro- vides evidence that there is a characteristic distribution and evolution of AChE staining within cytoarchitectonic compartments of the developing human auditory cortex The early bilaminar pattern of AChE staining (marginal zone and subplate layer) corresponds to the bilaminar distribution of synapses (Molliver Kostović & Van der Loos 1973 Kostović Knežević, Kostović Krmpotić Nemanić Ke- lović & Vuković, 1978) observed in human fetal cortex Thus AChE-positive fibres may be involved in early fetal synaptogenesis The onset of AChE staining within the cortical plate at 24 weeks of gestation corresponds to the appearance of synapses in this layer (Molliver Kostović & Van der Loos 1973 Kostović & Molliver 1974) and may indicate the establishment of thalamocortical circuitry In accordance with this hypothesis is our finding of AChE-rich, vertically oriented al ternating zones in prospective layer IV of the auditory cortex at 28 weeks of gestation This columnar pattern of AChE staining which has been also observed by us in frontal cortex of human fetus (Kostović 1979) may correspond to the columnar distribution of thalamocortical fibres found in primate neocortex (Hubel & Wiesel 1969 Rakic 1976) or granule cells in primate auditory cortex (Smith & Moskowitz, 1979) while AChE-poor zones may corre spond to the termination of cortico-cortical fibres observed in the frontal cortex by Goldman & Nauta (1977) Vertically oriented AChE-positive bands may be also related to the columnar cortical organization identified by both physiological and neuroanatomical approaches (Mountcastle 1957 Hubel & Wiesel 1977 Hubel Wiesel & Stryker 1978) Thus our observations (Kostović 1979· this study) may constitute the first evidence of columnar organization in the human cerebral cortex.

As suggested previously (Vaughan 1975 Krmpotić Nemanić, Kostović Nemanić & Kelović, 1979) the early establishment of the main organization of the auditory cortex may be of essential significance for both normal and abnormal development of the central audi- tory system

At present we have no evidence that the AChE-positive reaction found in our material may reflect the cholinergic nature of the tha lamic input to auditory cortex as was proposed for other sensory cortices (Kristt 1979) It is possible that the presence of AChE is related to the process of growth and differentiation and may be present in some developing telen cephalic fibre systems (Kostović Knežević, Kostović Krmpotić Nemanić & Kelović 1979· Kostović Kelović Kračun & Krmpotić Nemanić 1979) Furthermore the AChE positive reaction may be related to some non- cholinergic inputs as suggested by Emson & Lindvall (1979)

RÉSUMÉ

Dans cette étude les méthodes de coloration avec acetyl- cholinesterase (AChE) étaient adoperées pour demontrer le development de fibres afférientes du future cortex auditif dans les fetus humains de 8 à 28 semaines de gestation. La première AChE positive reaction apparait chez les fetuses de 20–24 semaines dans le neuropil de la zone marginale et dans le subplate layer du cortex auditif Par cette coloration charactéristique avec AChE le corte auditif peut être delimité des autres regions corticales. A 24–26 semaines pendant la lamination ul- terieure de la zone corticale AChE-positiv reaction fut trouvée dans la partie perfonde de la zone corticale Dans les stades de 28 semaines une structure en colonnades verticale et sombre dans le tiers intermediaire de la zone corticale fut observée On peut en conclure que les fibres

AChE positives ont une distribution charactéristiques dans les compartiments dytoarchitectoniques et couches synaptique du cortex auditif en development Les couches AChE positives coincident avec la structure laminaire de la synaptogenèse Cette reaction pendant le developpment peut servir comme indicateur de l'innervation corticale afférente (thalamique) du cortex auditif Cette reaction pourrait indiquer la nature spécifique du médiateur des fibres afférentes dans le cortex auditif

ZUSAMMENFASSUNG

In dieser Studie wurden die Acetylcholinesterasefärbungmethoden (AChE verwnedet um die Entwicklung der prospektiven afferenten Fasern des auditiven Cortex in menschlichen Feten von 8–28 Schwangerschafts wochen zu demonstrieren. Erste AChE-positive Färbung wurde bei Feten in 20 – 4 Woche im Neuropil der marginalen Zone und im „subplate layer" *des auditiven Cortex* gefunden Durch diese charakteristische AChE Färbung kann der auditive Cortex von den umgebenden cortikalen Flachen underschieden werden In den 4 – 6 Wochen während der intensiven Lamination der Cortikalplatte wurde die AChE-positive Färbung in der tiefen Schicht der Cortikalplatte gefunden In den älteren Stadien (?8 Schwangerschaftswochen) wurde eine säulenförmige Struktur aus vertikalen sich dunkel farbenden Zonen im mittleren Drittel der Cortikalplatte gefunden Wir können mit der Feststellung schlißen daß die AChE Färbung in den cytoarchitektonischen Flachen und „synaptischen Schichten" des sich entwickelnden auditiven Cortex charakteristisch verteilt ist Die AChE-positive" Schichten fallen mit dem laminären Muster der Synaptogenese zusammen so daß diese Reaktion wahrend der Entwicklung als Parameter der cortikalen afferenten (thalamischen) Innervation des auditiven Cortex dienen kann. Die AChE Reaktion könnte auf eine spezifische Transmitterart der afferenten Fasern des auditiven Cortex hinweisen

ACKNOWLEDGEMENTS

We thank Mrs Zdenka Cmuk Mrs Danica Nežić and Mr Tiburcije Pill for their excellent technical assistance

This work was supported by a SIZ V (SRH) Research grant (Agreement V 10/1978)

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J Krmpotić Nemanić
Dept of anatomy
Medical Faculty
Šalata 11 POB 286
4100 Zagreb
Yugoslavia

Acta Otolaryngol 89 393–399 1980

DIE BEDEUTUNG DER ZELLPROLIFERATION UND HISTOMORPHOLOGIE BEI DER GEPLANTEN BEHANDLUNG DER KEHLKOPFKARZINOME

J. Élő

Aus der HNO Klinik des Instituts für ärztliche Fortbildung, Budapest, Ungarn

Inhalt. Die Differenzierungsmöglichkeit der einzelnen Phasen des Zellzyklus und die Kenntnisse über Populationskinetik der Gewebe erwiesen sich als bedeutend zur besseren Erkennung des biologischen Benehmens der Tumore. Neben den klinischen Eigenschaften (Lokalisation, TNM Stadium) und des Onkotyps der Geschwulst sind auch die zellkinetischen Parameter sehr wichtig. Es wurde bei Kehlkopfkarzinomen der Zusammenhang zwischen dem Malignitätsgrad und den zellkinetischen Daten untersucht. Aus der Bestimmung des Markierungsindex (L.I.) und der DNA Synthesezeit sind auch die übrigen populationskinetischen Angaben zu erfinden. Es konnte bei der autoradiographischen Untersuchung von 78 Kehlkopftumoren ein Zusammenhang zwischen der Grösse des L.I. und dem biologischen Benehmen des Tumors gefunden werden. Wir konnten bei den wenig differenzierten oder anaplastischen Karzinomen immer höhere L.I. feststellen. Zusammen mit den klinischen Angaben des Karzinoms weist der erhöhte L.I. auf die Möglichkeit der häufigeren Metastasierung hin. Auf Grund der zellkinetischen Daten ergibt sich eine bessere differenzierte Therapie. Man kann auf die Wirksamkeit der präoperativen Strahlen- und Zytostatischen Behandlung folgen.

In den vergangenen 3 Jahrzehnten wurde wiederholt über die Bedeutung der Proliferationskinetik normaler und maligner Gewebe berichtet.

Zugrunde liegt die Unterteilung des Zellzyklus nach Howard & Pelc in die intermitotische (G_1 – S – G_2) und mitotische (M) Phase und die Unterteilung der Zellpopulation in die proliferierende und nichtproliferierende Fraktionen – nach Mendelsohn (Growth fraction).

Proliferative Eigenschaften der Geschwülste erwiesen sich als bedeutend zur besseren Erkennung des biologischen Benehmens maligner Tumoren, deshalb untersuchten wir – zusammen mit den histomorphologischen Eigenschaften der Larynxtumoren – die Bedeutung der Zellkinetik.

MATERIAL UND METHODE

Zu den zellkinetischen Untersuchungen wurde ^{3}H Thymidin (^{3}H[TdR]) angewandt.

Mit in vivo und in vitro Incorporation der Radionucleosid bestimmten wir in 7 Fällen die Zeit der DNS-Synthesephase (Ts) und die Werte des Markierungsindex (L.I.)

Vor der Biopsie spritzten wir direkt in den Tumor 30 μCi ^{3}H[TdR] bei den leicht zugänglichen supraglottischen Geschwülsten. (Die Lösung wurde mit Toluidin-blau gefärbt.) Nach 30 Minuten geschah die erste Exzision. Das Gewebestück wurde histologisch und autoradiographisch aufgearbeitet. Zwei Stunden später folgte die zweite Probeexzision aus dem markierten Gebiet, dann wurde noch für 30 Minuten in eine auf 37°C temperierte α MEM (minimum essential medium) inkubiert. Die Lösung enthielt 5 μCi/ml ^{3}H[TdR] und wurde mit Oxygen durchgeströmt. Anschliessend erfolgte die Fixierung und Einbettung. Die Schnitte wurden entparaffiniert und mit Ilford G-5 Emulsion bedeckt. Nach -wöchiger Expositionszeit wurden die Schnitte entwickelt, fixiert und mit Hämotoxylin-Eosin (H.E.) gefärbt.

Aus dem Unterschied der L.I. Werte der in vivo – in vitro markierten und nur in vivo markierten Preparaten ist es möglich die Zellströmung bzw. den Fluxus der DNS-synthetisierenden Zellen zu bestimmen. (An Hand von der Methode von Galand, Lala und Muggia.)

Auf der 1. Abbildung sind die vereinfachten Formeln zu sehen. Mit Hilfe dieser Gleichungen kann der L.I. Der Mitoseindex (M.I.) aus dem Zellfluxus während einer Stunde die

1 $LI = \frac{NS}{N} \cdot 100$ 3H MARKIERUNGSINDEX LABELLING INDEX

2 $MI = \frac{Nm}{N} \cdot 100$ MITOSEINDEX

3 $\Delta LI = \frac{LI\,(\text{in vivo} + \text{in vitro}) - LI\,(\text{in vivo})}{t}$

ΔLI = ZELLFLUXUS WÄHREND EINER STUNDE

4 $T_S = \frac{LI\,(\text{in vivo})}{\Delta LI}$ DNS SYNTHESEZEIT

5 $T_C \simeq T_S \times 3\text{–}4$ GENERATIONSZEIT

6 $PR = \frac{T_S}{T_C} LI$ PROLIFERATIONSRATE PR = GF GROWTH FRACTION

7 $DT_{POT} = \alpha \frac{T_S}{LI}$ POTENTIELLE VERDOPPELUNGSZEIT α = GROWTH CONSTANT (STEEL)

8 $\Phi = 1 - \frac{DT_{POT}}{DT}$ ZELLVERLUST (CELL LOSS)

Abb 1 Formeln, die im Vortrag vorkommen

Ts Zeit bestimmt werden Mit Bezugnahme auf die Publikationen von Fabricant Frindel Kissel Klein Schultze Steel Terz Young kann die Tc 3–4 mal länger als die Ts angesehen werden

An Hand der zellkinetischen Angaben sind die populationskinetischen Parameter die Grösse der proliferierenden Fraktionen (G.F und die potentielle Verdoppelungszeit (T.I pot) zu bestimmen (Steel)

Mit Hilfe der Beobachtungen der Patienter die nach der Probeexzision monatelang jeder lei Behandlung zurückweisen ist es möglic die Zunahme des Tumors zu beurteilen Daraus kann die klinische Verdoppelungszei (D T) ausgerechnet werden In Abhängigkei der klinischen und potentiellen Verdoppelungszeiten ist die Grösse des Zellverluste (Cell loss) zu bestimmen (Steel)

Eine der wichtigsten zellkinetischen Angaben ist der L I (Fabrikant) Dieser Parameter wird aus dem Biopsiematerial mit in vitro ^{3}H[TdR] Markierung festgelegt (Help Nitze Rajewsky)

Mit diesem Verfahren kann man mit Sicherheit die in dem gegebenen Zeitpunkt DNS synthetisierenden Zellen markieren wobe auch wichtig ist dass es keine extra Belastung oder Gefahr für die Patienten bedeutet Zwischen 1974–1978 wurde der L.I bei 7 Kranken bestimmt

Gleichzeitig wurde auch der Mitoseindex (M I) festgelegt Das Geschwulstgewebe wurde sofort ohne Inkubation fixiert histologisch aufgearbeitet und mit H E oder nach Feulgen gefärbt

Tabelle I *Nach in vivo–in vitro Incorporation von ^{3}H[Thymidin] bestimmte Markierungsindices und DNS Synthesezeit bzw geschätzte zellkinetische Parameter der Larynxkarzinome*

	Differenzierungsgrad						
	Hoch-differenziert			Mittelmassig (moderately) diff		Gering (poorly) diff	
Zahl der Fälle	3			2			
^{3}H[TdR] Markierungsindex in vivo L.I	5 0	6,3	7,5	7,8	9 6	10 1	13,–
H[TdR] Markierungsindex in vivo + in vitro (^{3}H) L I	5 7	6,84	8 4	8,9	10,8	11,36	14,6
Zellfluxus/Stunde/ ΔL I	0,35	0,27	0 45	0,55	0 6	0,63	0 71
	7 %	4,3 %	6 %	7 %	6,3 %	6,2 %	5 4 %
DNS-Synthesezeit S-phase	14,3	23,3	16 6	14,2	16,0	16 0	18,6
Generationszeit TC		63		53		61	
Proliferationsrate P.R G F		0,22		0,31		0 41	
Pot Verdoppelungszeit DT POT		188 (1)		174 (7,3)		148 (6,–)	
Akt Verdoppelungszeit		~180 Tag		~150 Tag		~120 Tag	
Zellverlust, Φ		93 %		95 %		95 %	

Tabelle II ³H Thymidin Markierungsindex und die histopathologischen Angaben der Larynx Karzinome 1974–1978

	Differenzierungsgrad		
	Hochdifferenziert	Mittelmässig (moderately) diff	Gering. differenziert und undifferenzierte
Cases	29	31	18
³H Markierungsindex, L.I %	(5–7,8) 6,5	(7,– 10,5) 8,5	(9,8–15,1) 11,9
Mitoseindex, M.I %	(0,7–1,3) 1,06	(0,9–1,1) 1,43	(1,5–.,4) 1,91
Zellkern-Morfologie (nuclear grading)	NG 3	NG 3–NG	NG 1
Necrosis formation	0 +	+	+
(an der Randzone)	Umgrenzend	Gering-tief (irregular)	Tief infiltrativ (highly)

Es sei betont, dass der M.I. inkubierten Gewebes immer geringer als *in vivo*-Verhältnissen ist, weil die im Gang befindlichen Mitosen während der Inkubation ablaufen, aber neue Zellen aus der G_2-Phase in die M Phase nicht übertreten (Rajewsky, Oehlert).

ERGEBNISSE

Auf Grund des histologischen Differenzierungsgrades sind die Kehlkopfpatienten in 3 Gruppen eingeteilt worden:

- in der I. Gruppe: Kranken mit hochdifferenzierten,
- in der II. Gruppe: Kranken mit mittelmässig differenzierten,
- in der III. Gruppe: Kranken *mit gering* differenzierten Karzinomen.

Dem Reifegrad entsprechend wurden auch die Zell- und populationskinetischen Parameter gruppiert.

Die Tabelle I zeigt die mit in vivo und in vitro ³H[TdR] Markierung bestimmten und geschätzten Werte.

Die Werte der Markierungsindices sind innerhalb einer Gruppe auch nicht gleich, trotzdem kongruieren sie mit dem Differenzierungsgrad des Tumorgewebes gut.

Die Wachstumfraktion (G.F.) erweist sich als eine andere charakteristische Angabe. Mit Abnahme der Ausreifung der Tumorzellen steigt die Zahl der proliferierenden Zellen des Gewebes. Es entspricht der von Stein abgefasster Grundregel: mit der Abnahme der strukturellen Differenzierung nimmt die funktionelle Kapazität ab und es steigt die reproduktive, proliferative Kapazität des Gewebes.

Der Zellverlust beträgt mehr als 90% stammt aus Zelltod. Migration und die Exfoliation spielt bei den Larynxtumoren eine wesentliche Rolle.

Die nach in vitro ³H[TdR] Incorporation bestimmten Angaben von 78 L.I. wurden nach dem strukturellen Differenzierungsgrad gruppiert. Die Ergebnisse sind in der II. Tabelle dargestellt.

Die Werte der Markierungsindices sind mit dem histopathologischen Befunden in gutem Einklang und ändern sich zwischen 5–15%. Ein L.I. der unter 8% liegt, ist für die gut differenzierten Geschwülsten charakteristisch. Der L.I. wurde nur dann über 10% gefunden, wenn das Karzinom geringdifferenziert oder undifferenziert war.

Mit den anderen morphologischen Kennzeichen der Malignität des Tumors zeigte der L.I. eine gute Korrelation.

Die Zellkernatypie ist bei den hochdifferenzierten Geschwülsten nach Bloom und Black (NG3) geringer, aber mit der Abnahme des strukturellen Reifegrades können sowohl in Form, als auch in der Grösse und Färbung bedeutende Unterschiede gefunden werden (Pleomorphismus NG² NG1).

Die Ausdehnung der Nekrose bei nicht

1 $L\,I = \frac{NS}{N}\,100$ ³H MARKIERUNGSINDEX LABELLING INDEX

2 $M\,I = \frac{Nm}{N}\,100$ MITOSEINDEX

3 $\Delta L\,I = \frac{L.I.(\text{in vivo} + \text{in vitro}) - L.I.(\text{in vivo})}{t}$

$\Delta L\,I$ = ZELLFLUXUS WÄHREND EINER STUNDE

4 $T_S = \frac{LI(\text{in vivo})}{\Delta L\,I}$ DNS SYNTHESEZEIT

5 $T_C \approx T_S \times 3{-}4$ GENERATIONSZEIT

6 $PR = \frac{T_C}{T_S}\,L\,I$ PROLIFERATIONSRATE PR GF GROWTH FRACTION

7 $DT_{POT} = \alpha\,\frac{T_S}{L\,I}$ POTENTIELLE VERDOPPELUNGSZEIT α = GROWTH CONSTANT (STEEL)

8 $\Phi = 1 - \frac{D.T_{POT}}{D.T}$ ZELLVERLUST (CELL LOSS)

Abb 1 Formeln die im Vortrag vorkommen.

Ts Zeit bestimmt werden Mit Bezugnahme auf die Publikationen von Fabricant frindel Kissel Klein Schultze Steel Terz Young kann die Tc 3–4-mal länger als die Ts ange sehen werden

An Hand der zellkinetischen Angaben sind die populationskinetischen Parameter die Grösse der proliferierenden Fraktionen (G F) und die potentielle Verdoppelungszeit (T.D pot) zu bestimmen (Steel)

Mit Hilfe der Beobachtungen der Patienten, die nach der Probeexzision monatelang jeder lei Behandlung zurückweisen ist es möglich die Zunahme des Tumors zu beurteilen. Daraus kann die klinische Verdoppelungszeit (D T) ausgerechnet werden In Abhängigkeit der klinischen und potentiellen Verdoppelungszeiten ist die Grösse des Zellverlustes (Cell loss) zu bestimmen (Steel)

Eine der wichtigsten zellkinetischen Angaben ist der L.I (Fabrikant) Dieser Parameter wird aus dem Biopsiematerial mit in vitro ³H[TdR] Markierung festgelegt (Helpap, Nitze Rajewsky)

Mit diesem Verfahren kann man mit Sicherheit die in dem gegebenen Zeitpunkt DNS-synthetisierenden Zellen markieren wobei auch wichtig ist dass es keine extra Belastung oder Gefahr für die Patienten bedeutet. Zwischen 1974–1978 wurde der L.I bei 78 Kranken bestimmt

Gleichzeitig wurde auch der Mitoseindex (M I) festgelegt Das Geschwulstgewebe wurde sofort ohne Inkubation fixiert histologisch aufgearbeitet und mit H E oder nach Feulgen gefärbt

Tabelle I *Nach in vivo–in vitro Incorporation von ³H[Thymidin] bestimmte Markerungsindices und DNS Synthesizeit bzw geschätzte zellkinetische Parameter der Larynxkarzinome*

	Differenzierungsgrad						
	Hoch-differenziert			Mittelmässig (moderately) diff		Gering (poorly) diff	
Zahl der Fälle	3			2			
³H[TdR] Markierungsindex in vivo L.I	5 0	6,3	7,5	7,8	9 6	10 1	13,2
H[TdR] Markierungsindex in vivo + in vitro (**) L I	5 7	6,84	8 4	8,9	10,8	11,36	14,6
Zellfluxus/Stunde/	0,35	0,27	0 45	0,55	0,6	0,63	0,71
ΔL I	7 %	4,3 %	6 %	7 %	6,3 %	6,_ %	5 4 %
DNS-Synthesezeit S-phase	14,3	23,3	16 6	14,2	16,0	16 0	18,6
Generationszeit TC	63			53		61	
Proliferationsrate P.R G F	0,22			0,31		0 41	
Pot. Verdoppelungszeit DT POT	288 (12)			174 (7,3)		148 (6,_)	
Akt Verdoppelungszeit	~180 Tag			~150 Tag		~120 Tag	
Zellverlust, Φ	93 %			95 %		95 %	

Tabelle IV *TNM System (UICC) des Kehlkopfs ergänzt mit dem Differenzierungsgrad und H Markierungsindex der Geschwülste*

D_1 Hoch–Mittelmässig differenzierte Geschwülste L.I <9
D_2 Geringdifferenzierte und ana-plastische Geschwülste L.I. >9

Stadium	T	N	N	V N M	D
Stadium	T	N_{0}	N_{1a}	V N_{0} M_0	D_1 D_2
I Stadium	T_{1}	N_{0}	N_{1a}	V N_{0} M_0	D_1 D_2
II Stadium	T_{1} T T T_{2}	N_{0} N_{0} T T	N_{1a} N_{1a} N_{1a}	V N_{0} M_0 M_0 V N M_0	D_1 D_2
IV Stadium	T T N_{0}	T_{1} T N	T T N_3	V T_4 N_3 M_0 V T M V N	D_1 D_2

...chungen von 78 Kehlkopftumoren konnte ein Zusammenhang zwischen der Grösse des L.I und dem biologischen Benehmen des Tumors gefunden werden. Wenn der L.I eines Karzinoms unter 8% ist, ist sein Malignitätsgrad niedrig. Über 10% ist der Malignitätsgrad des Tumors hoch. Wir fanden bei den wenig differenzierten oder anaplastischen Karzinomen immer hohe Markierungsindices und proliferierende Fraktionen.

Zusammen mit den klinischen Angaben des Tumors weist der Onkotyp und die mitotische Aktivität auf die Häufigkeit der Metastasen hin (Bauer).

Je mehrere Angaben in einem konkreten Fall bekannt sind, desto sicherer kann man die prognostischen und therapeutischen Folgerungen ziehen. Alderson fand bei Mammatumoren, Ferlito bei Larynx- und Hypopharynxtumoren die multifaktorielle Analyse als ergebnisvoll.

Die 2. Abbildung zeigt unseren Standpunkt bei der Beurteilung der Larynxtumoren.

Mit Hilfe der histologischen und zellkinetischen Angaben ergänzten wir das von UICC eingeführte TNM System mit dem Differenzierungsgrad und L.I des Larynxkarzinoms.

Mit D_1 wurden die hoch- oder mittelmässig differenzierten Karzinome mit einem L.I under 9% bezeichnet.

Mit D_2 wurden die gering oder undifferenzierten Karzinome mit einem L.I über 9% bezeichnet.

So ergab sich die Möglichkeit, dass die in dasselbe Stadium gehörenden Kranken doch in 2 Gruppen separiert sein konnten.

59 von den erwähnten Patienten haben schon mindestens 3-jährige Behandlungsergebnisse. Ein signifikanter Unterschied konnte zwischen der D_1 and D_2 Gruppe festgestellt werden.

Tabelle V *3-Jährige Überlebensrate auf Grund der TNM Stadien, Differenzierungsgrad und H Markierungsindex der Larynxkarzinome (D und D_2)*

I Stadium	II Stadium		III Stadium		IV Stadium	
	D	D_2	D	D_2	D_1	D_2
Kein Autoradiogramm	0/7 100%	2/6 67%	2/22 91%	6/15 60%	2/6 67%	2/2 0%

Insgesamt D_1 = 4/35, 89% D_2 10/24, 58%

Table III *Zusammenhang zwischen histologischer Klassifikation + ^3H[TdR]Markierungs index und der klinischen Angaben der Larynxkarzinome*

	Malignitätsgrad		
	I Gering (low)	II Übergangsstadium (Intermediate)	III–IV Groß (high)
Casus	29	31	18
H Markierungsindex L.I	6,5	8,5	11 9
Wachstum	65 % Exophyt	Exophyt–Endophyt	Endophyt
Tastbare Lymphknoten vor der Operation	5/29 17 %	15/31 48 %	13/18, 72 %
Überlebensrate (3-jährig)	2/21 90 %	3/25 88 %	9/13 31 %

behandelten Karzinomen ist mit der Proliferationsgeschwindigkeit im Verhältnis (je schneller desto grösser)

Das Bild des Marginalgebietes zwischen den normalen und malignen Gewebe zeigt sich auch als charakteristisch Die Tiefe der *Invasion* und besonders *des Grad der Rund ell infiltration* sind von grosser Bedeutung Es wurde der Zusammenhang zwischen dem L I und den klinischen Angaben untersucht

Auffallend und wichtig ist die Beziehung zwischen dem L.I und dem Vorkommen der vor der Operation vergrösserten Lymphknoten Mit niedrigen L I fanden wir selten Lymphnoden dagegen wenn der L I über 10 % war konnten wir sehr oft Lymphknoten

A KLINISCHE EIGENSCHAFTEN
1 LOKALISATION
2 TNM KLASSIFIKATION
3 MAKROSKOPISCHE ASPEKTE

B HISTOMORFOLOGISCHE EIGENSCHAFTEN
1 ONKOTYP
2 MALIGNITATSGRAD (I II)

C IMMUNMORFOLOGISCHE EIGENSCHAFTEN
1 MARGINALE UND INTRATUMORALE RUNDZELL – INFILTRATION
2 REAKZIONSTYP (CELLULAR HUMORAL) DER VERGRÖSSERTEN LYMPHKNOTEN

D ZELLKINETISCHE PARAMETER

Abb 2 Faktoren die bei uns die Therapie der Larynxkarzinome bestimmen

tasten Die Metastasengefahr ist bei den schnellproliferierenden Tumoren viel häufiger Dies stimmt mit den Beobachtungen von Arthur McGavran und Sessions zusammen die einen signifikanten Zusammenhang zwischen der Vorkommen der Lymphknotenmetastasen und dem *strukturellen Reifegrad* der Tumoren fanden

DISKUSSION UN THERAPEUTISCHE FOLGERUNGEN

Neben den klinischen Eigenschaften des Kehlkopfkarzinoms (Sitz, Grösse Metastasen sind die histologischen zellkinetischen Parameter und die Immunkapazität des Organismus von grosser Bedeutung Obwohl das TNM System sich als nützlich beweist ist eine an breiter Basis ruhende Klassifikation notwendig um die Proliferationsaktivität und die Aggressivität des Tumors zu beurteilen und um einen besseren Therapieplan finden zu können

Es wurde bei Kehlkopfkarzinomen ein enger Zusammenhang zwischen dem Malignitätsgrad (struktureller Differenzierung Pleomorphismus der Zellen Nekrosis Invasion und Mitosenzahl) und dem zellkinetischen Parameter gefunden Aus der Bestimmung des L I und des TS sind auch die populationskinetischen Angaben zu erfinden (potentielle Verdoppelungszeit proliferierende Fraktion Zellverlust)

Bei den autoradiographischen Untersu-

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Dr J Élő
H Budapest
P.O Box 112
Hungary

Bei den D_2 Tumoren ergab sich die 3-jährige Überlebensrate um 31 % niedriger als bei den D_1 Tumoren Daraus muss eine wichtige Kon sequenz gezogen werden die Kranken die einen geringdifferenzierten und schnell proliferierenden Tumor haben befinden sich in grösserer Gefahr und brauchen eine viel seitigere und gründlichgeplante Therapie In diesen Fällen sind die rationellen Kombina tionsmöglichkeiten der chirurgischen Strah len- und zytostatischen Behandlungbesonders wichtig

Es ist schon seit langem bekannt dass eine Korrelation zwischen der Proliferations aktivität und der Radiosensibilität besteht (Bloom Coutard Fletcher Rubin) Je schnel ler das Wachstum ist desto grösser ist die Wirkung der ionisierenden Strahlen Die schneller proliferierenden und geringdifferen zierten D_2 Larynxkarzinome sind mit grösse rer Wahrscheinlichkeit radiosensibler darum bekommt die Bestrahlung bei diesen Kranken eine wichtige Rolle

In Abhängigkeit von den Ergebnissen der multifaktoriellen Analyse wurde unser Thera pieplan zusammengestellt wie folgt

I Bei der Klasse T_1
  1 In der Gruppe D_1 Operation bevorzugt
  2 In der Gruppe D_2 Strahlentherapie

II Bei der Klasse T_2
  1 In der Gruppe D_1 Operation bevorzugt
  2 In der Gruppe D_2 Strahlenbehandlung bis 4000 rad dann eine Woche Pause

Je nach der Tumorrückbildung wird ent weder die Irradiation bis zu einer Gesamt dosis von 6000 rad fortgesetzt oder operiert

III Bei der Klasse T_3
  1 In der Gruppe D_1 chirurgischer Ein- griff ohne Bestrahlung
  2 In der Gruppe D_2 kombinierte Therapie

Nach 3000 rad folgt innerhalb einer Woche die chirurgische Massnahme Nach der Hei lung wird die Radiotherapie komplettiert Bei ausgedehnten Tumoren halten wir die Totalexstirpation für die Methode der Wahl

IV Bei der Klasse T_4
Immer kombinierte Behandlung Nach 6000 rad Gesamtdosis folgt eine 4-wöchige Pause dann der chirurgische Eingriff

Besonders in dieser Gruppe können die Strahlensensibilitätmodifizierenden Faktore (Zytostatika Metronidasol-Derivate, usw) zur Anwendung gebracht werden (Chapman Yuhas)

SUMMARY

The separation of phases in cell division and the recog nition gained in tissue population kinetics have con tributed to enhance our knowledge of the biological behaviour of the tumours. Besides the determination of clinical characteristics (localization, TNM state) and the onchotype of a given tumour findings obtained by cell-kinetic parameters seem to be of great importance Relations between the degree of malignity and the data obtained by autoradiography were studied in patients suffering from laryngeal cancer On the basis of the simple clinical determination of L.I and Ts estimation of further cell-kinetic parameters in a given tumour has become possible and the number of cells in the same cycle the growth faction and the potential doubling time can be deduced After the radiographic investigation of 78 cases of laryngeal tumour a relation was found be tween the extent of the labelling index and the biological behaviour of the tumour

RÉSUMÉ

La séparation des phases de division cellulaire et la reconnaissance de la kinétique de la population des tissues ont contribué considérablement à ce que nous connais sons mieux l'attitude biologique des tumeurs. A côté des caractéristiques biologiques et cliniques (la localisa tion le stade en TNM) et la définition du type onkotique, il est très important de connaître les paramètres de la kinétique cellulaire Nous avons examiné le degré de la malignité chez des malades au cancer du larynx et la rélation de cela aux informations reçues par la méthode autoradiographique La technique de la définition de L I et T S est facile et donne la possibilité pour calculer des paramètres de la kinétique cellulaire d'un tumeur donné A la base des données obtenues par la kinétique cellulaire nous avons la possibilité d'un traitement plus differentiel dans des cas du tumeurs du larynx et nous pouvons y tirer des conclusions concernant l'éfficacité d'un traitement cytostatique et d'une irradiation préoperative

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Acta Otolaryngol 89: 401–406 1980

STREPTOMYCIN INDUCED DEFECTS OF THE OTOCONIAL MEMBRANE

Lars-Göran Johnsson, Charles G Wright, Robert E Preston and Pamela J Henry

From the Kresge Hearing Research Institute, Department of Otorhinolaryngology, University of Michigan Medical School, Ann Arbor, Michigan, and from the Callier Center for Communication Disorders, The University of Texas at Dallas, Dallas, Texas, USA

(Received March 23 1979)

Abstract. Damage to the neuro-epithelium caused by the vestibulotoxic drugs has been well described in previous reports, however little is known about the effect of such drugs on the otoconia. In this investigation, streptomycin sulfate was found to create a circumscribed defect in the crystalline layer of the otoconial membrane in the utricle of the guinea pig. The defect was secondary to a sharply outlined lesion of the neuroepithelium which corresponded in size and location to the otoconial defect. Many of the otoconia along the margin of the defect showed signs of degeneration and appeared similar to those observed in the saccule of man during aging. In advanced stages of decay the otoconia were reduced to hollow shells consisting of longitudinal interconnecting strands. All the epithelial lesions occurred with remarkable consistency in the same region of the macula utriculi at the posterior end of the striola.

The aminoglycosides streptomycin, gentamicin and tobramycin are the most important vestibulotoxic drugs. Their major site of action is the neuro-epithelium and the damage they cause has been well documented (Hawkins, 1976). This report deals with injury to the utricular otoconia caused by streptomycin.

After intratympanic application of streptomycin, the sensory epithelium of the macula utriculi shows less damage than the ampullary cristae and the macula sacculi still less (Lindeman 1969). It is of interest to note that the Type I hair cells located within the striola (Werner 1940) are more vulnerable than the Type II cells. Streptomycin is also known to affect the so-called dark cells in the membranous wall of the utricle (Hawkins & Preston, 1973).

Reports of otoconial abnormalities have been few. We have observed what probably represented abnormal saccular otoconia in cats after gentamicin (Hawkins et al. 1969) and neomycin (Johnsson & Hawkins 1977) treatment. Neomycin appeared to cause loss of otoconia on the posterior tip of the saccule while gentamicin created more generalized loss of saccular otoconia with some giant otoconia remaining. Because the specimens had been stored for several months in 70% alcohol the pH of which was not measured, the validity of these observations is open to question. Lim (1973) has observed abnormal otoconia in the guinea pig which he believes were altered due to ethacrynic acid treatment. Recently, loss of both saccular and utricular otoconia has been observed in guinea pigs after administration of streptomycin (Harada & Sugimoto 1977). As has been mentioned in our preceding report, experimentally induced *otoconial abnormalities* in guinea pigs have to be viewed in light of the fact that defective otoconial membranes do occur in untreated control animals (Johnsson et al. 1979).

This report deals with an otoconial defect which with certainty can be related to degenerative changes in the neuro-epithelium. The observation was made during dissection of the vestibular system in guinea pigs intended for use in a study of the effect of streptomycin on the incorporation of ^{45}Ca in the otoconial membranes.

METHODS

The technique of microdissection which was employed in the previous study (Johnsson et al. 1979) was also used in this investigation.

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METHODS

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and has been described in detail elsewhere (Hawkins & Johnsson 1975) The original purpose was to investigate the uptake of ⁴⁵Ca in the otoconial membranes an extensive morphological study was not done The specimens were fixed for approximately 24 hours in Zetterqvist s 1% osmium tetroxide solution and partially dehydrated in ascending concentrations of alcohol The cochlea and the ampullary cristae were not studied Most of the maculae were examined only in the dissection microscope Surface preparations of the neuro-epithelia were prepared from a few maculae and viewed in phase-contrast illumination The neuro-epithelia and the otoconial membranes of the utricular maculae from 4 guinea pigs were examined by scanning electron microscopy The otoconial membranes were air dried and the maculae were CO^2 critical point dried with amyl acetate as intermediate fluid

MATERIAL

Twenty five albino guinea pigs were treated with subcutaneous daily doses of streptomycin sulfate powder (Pfizer) dissolved in saline 200 mg/kg for 19 to 20 days The animals were killed at various intervals after the last drug dose Marked ataxia was seen in 8 of 25 streptomycin-dosed animals There was no correlation between the presence of ataxia and degree of depression of the pinna reflex A subsequent series of 18 similarly treated guinea pigs was examined The findings in this second group of animals were in all respects similar to those in the first group and will not be described in detail Untreated guinea pigs used in other studies (Mechigian et al 1979) served as control animals

RESULTS

Otoconial defects

A striking circumscribed loss of otoconia was observed in the posterior half of the macula utriculi in several animals No similar changes were found on the macula sacculi

Because the guinea pigs were killed at various intervals after the last streptomycin dose it was possible to observe what appeared to be different stages and growth of the lesion after termination of the drug administration. With a few exceptions the size of the lesion was directly related to the time interval between the last injection and the day of sacrifice. The largest lesions were found in guinea pigs killed 20 days after the last dose Lesions were present in all groups except in the animals killed 2 days after the injection their neuro-epithelia and otoconial membranes all appeared normal under the dissection microscope

The large defects were characterized by complete loss of otoconia in the middle of the lesion which left the gelatinous part of the membrane uncovered and translucent (Johnsson & Hawkins 1967) This exposed the darkly stained macular tissue under the defect (Fig 1) The gelatinous layer of the otoconial membrane appeared normal The defects had an irregular shape and many otoconia at the margins showed varying degrees of staining probably due to demineralization

Examination of the margins by scanning electron microscopy revealed that some of the otoconia were in the process of degeneration These crystals appeared remarkably similar to the degenerated otoconia observed in the human saccule in association with aging (Ross et al 1976) Their surfaces were uneven with pitting and small grooves running longitudinally In advanced stages of decay the otoconia were hollowed out leaving a shell of longitudinal interconnected strands The end facets of the otoconia were less involved In advanced cases the otoconia were split into two or three fragments (Fig 2)

In guinea pigs killed 6 days after the last dose the changes in the otoconial membrane and the neuro-epithelium were even more circumscribed than in the other groups of animals and limited to a small round area The otoconial membrane at this point formed a dome over the epithelial defect creating a small cavity between the defect and the underside of

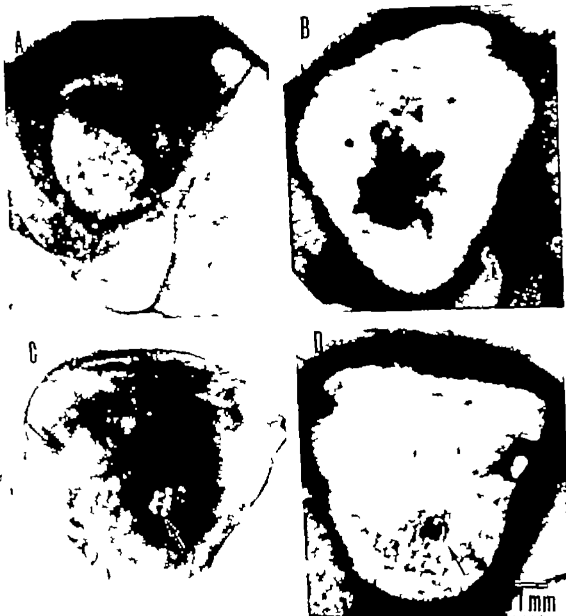


Fig. 1. Utricular maculae from two different animals. To the left the otoconial membrane has been dissected away to display the large epithelial defects. Note the close correlation in location and size between the epithelial lesions and defects in the otoconial membranes (shown to the right). *A, B*: large defects occurring 20 days after termination of streptomycin treatment (*left ear*). *C, D*: small lesion (*arrows*) seen close to the posterior tip of the macula 6 days after treatment (*right ear*). OsO

the membrane. There was no substantial loss of otoconia. Only a few minute holes in the otoconial layer surrounded by darkly-stained otoconia were seen at the top of the dome.

Epithelial defects

In every specimen in which otoconial changes were present, we found an epithelial defect, which corresponded in location and size to the defect in the otoconial layer. Often the epithe





Fig 2 Scanning electron micrograph of otoconia located along the margin of a large defect seen 20 days after treatment. Different stages of otoconial degeneration are seen Note the hollowed out otoconia with a shell of longitudinal strands holding together the more solid-appearing ends of the crystals Note also pitting and longitudinal fissures in earlier stages of degeneration (*arrows*).

lial defect was slightly larger than the involved area on the otoconial membrane *In a few* specimens there were two small separate epithelial defects (Fig 3) In those instances there were also two separate lesions on the otoconial membrane The epithelial defects appeared as if they had been punched out The larger lesions had thickened margins and were surrounded by several small satellite lesions The tissue exposed by the defects was not covered by an epithelium In some cases the neuroepithelium in the anterior part of the macula was loosened from the underlying tissue and formed a wide bulge These changes did not appear to result from artifacts and it is noteworthy that the otoconial layer above this particular region of damaged epithelium appeared completely intact under the dissection microscope

All epithelial defects were located with remarkable consistency in the same area of the posterior half of the macula on the striola This was especially clearly demonstrated in specimens with smaller lesions The characteristic location is shown in Fig 1 In the samples studied the sensory cell loss was in general most severe in the striola

Animals with defective otoconial membranes

In a previous study we found a high incidence of abnormal otoconial membranes in the utricle and saccule of untreated seemingly normal animals (Johnsson et al 1979) Abnormal specimens showing a marked reduction of otoconia over the striola could easily be identified in both the treated and untreated guinea pigs used in the present investigation In the animals with abnormal otoconial membranes streptomycin treatment produced epithelial defects identical with those described above When the epithelial lesion was larger than the pre-existing otoconial defect loss of otoconia could be observed which

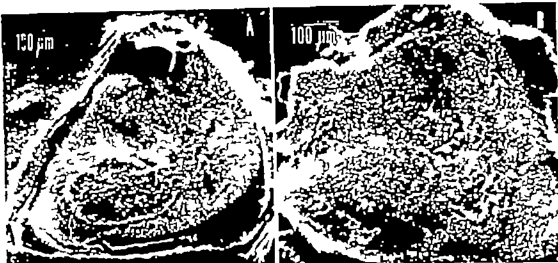


Fig 3 Scanning electron micrographs of the right utricular maculae from 2 animals killed 20 days after treatment. The tufts of sensory cells are displayed. The posterior tip of the macula is in the lower left and right corners respectively *A*, Single large lesion. The margin of the defect is thickened (*vertical arrow*). *B* T small defect (*horizontal arrow*) Not the sensory cell los along the striola.

clearly accentuated the original defect in the otoconial membrane

DISCUSSION

This study has demonstrated a more or less precise local correlation between a defect in the crystalline layer of the otoconial membrane and a streptomycin-induced lesion in the neuro-epithelium of the macula utriculi in guinea pigs One can only speculate about the process which caused the loss of otoconia. It is highly improbable that purely mechanical factors were involved We believe it is more likely that the epithelial damage interfered in some way with the calcium metabolism of the otoconia. Recent studies (Preston et al 1975 Mechigian et al 1979) have provided evidence for active turnover of calcium in the otoconial membranes. Thus the streptomycin treatment may have disturbed biochemical mechanisms responsible for calcium transport or uptake Also during degeneration of the neuro-epithelium, lysis of cells probably occurred leading to the release of lysosomal products and enzymes which may have lowered the pH sufficiently to cause dissolution of the otoconia. Such products of cellular degeneration might in addition alter the organic matrix of the otoconia as well as the gelatinous portion of the otoconial membrane

Our findings show little similarity to the otoconial changes recently attributed to streptomycin treatment by Harada & Sugimoto (1977) The degenerating otoconia in our specimens did appear similar to those found in the human saccule during aging (Ross et al 1976) which suggests that a common process might be involved in production of the two seemingly different forms of otoconial degeneration

This study does not show whether loss of sensory cells and supporting elements alone unaccompanied by focal defects of the maculae can alter the otoconia It seems likely that this will occur with time (Mechigian et al 1979)

The marked loss of Type I sensory cells in the utricle was to be expected (Lindeman 1969) but the circumscribed lesion occurring consistently at the posterior end of the striola was not anticipated. This finding suggests the presence in the utricle of a *locus minoris resis*

tentiae for streptomycin much like that in the lower basal turn of the cochlea for most ototoxic drugs Both the epithelial and the otoconial defects are unusual and could serve as useful tools in future morphological and functional studies on the macular organs

ACKNOWLEDGEMENTS

This investigation was supported by USPHS Grants NS 11672 and NS 17706 and Program Project Grant NS 05785 and by a grant from the Leland Fikes Foundation to the University of Texas at Dallas subcontracted through the Aberrant Behavior Center Dallas Texas

ZUSAMMENFASSUNG

Vestibulotoxische Neuroepithelschäden nach Aminoglykosidbehandlung sind von früheren Autoren beschrieben worden doch weiß man wenig über die direkte Wirkung dieser Medikamente auf die Otolithen In dieser Untersuchung wurde festgestellt daß Streptomycin eine begrenzte Schädigung der Kristallschicht der Otolithenmembrane der Macula utriculi verursacht Diese Schädigung stimmte der Größe und Lage nach mit einem scharfdefinierten Defekt des Neuroepithels überein Viele Otokonien am Rande des Defektes zeigten eine Art Degeneration wie sie bei der Macula sacculi des alternden Menschen beschrieben worden ist Im fortgeschrittenen Degenerationsstadium waren die Otokonien ausgehöhlt, und bestanden aus länglichen miteinander verbundenen Strängen. Alle Defekte befanden sich mit bemerkenswerter Regelmäßigkeit an der gleichen Stelle der Macula utriculi d h am hinteren Ende der Striola.

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Lars-Göran Johnsson M.D
Kresge Hearing Research Institute
University of Michigan Medical School
Ann Arbor
MI 48109
USA

Acta Otolaryngol 89 407–418 1980

THE NATURE OF THE OTOTOXIC ACTIONS OF ETHACRYNIC ACID UPON THE MAMMALIAN ENDOLYMPH SYSTEM

I *Functional Aspects*

S K. Bosher

From the Ferens Institute of Otolaryngology The Middlesex Hospital Medical School University of London London England

(Received May 8, 1979)

Abstract. The endolymphatic changes produced by an intravenous injection of 60 mg kg^{-1} ethacrynic acid were followed for up to 120 min using conventional and ion-sensitive (Na^+ K and pH) microelectrodes in the rat. They were found to be caused by three distinct effects upon the endolymph system. Initially the drug completely inhibited the strial potential-producing and cation-transporting processes. Recovery began quickly and was rapid at first Then its rate declined considerably probably due to diminution in strial energy production of delayed onset and prolonged duration Coincident with these actions upon activ transport, there was decrease in the overall cation permeability of the endolymph system. This followed different time course and affected K much more than Na The findings also provided further information about the mechanisms responsible for the normal endolymphatic composition. Qualitatively similar results were obtained in subsidiary study on guinea pigs

Reports of both transient and permanent deafness due to the use of ethacrynic acid began to appear in 1965 shortly after it was introduced into clinical practice Since then its effects upon the inner ear have been studied extensively As a result, it has been established that the stria vascularis is a major site of action of the drug and that significant changes occur in the cochlear endolymph especially in the endocochlear potential However almost all of the investigations have been phenomenological in nature and comparatively little is known about the way ethacrynic acid produces the alterations which have been described.

One of its most important effects is a marked decrease in energy utilisation by the stria vascularis which suggests that substantial inhibition of the active transport systems has probably taken place (Thalmann et al 1973 Kusakari et al 1978) There is also some diminution in energy production but this is relatively slight at the time of the maximal inhibitory action although the situation later has not been examined. The only strial ion-transporting enzyme so far identified a Na K -activated ATPase is relatively insensitive to ethacrynic acid and not much affected in vitro by the strial concentrations which inhibit the production of the endocochlear potential in vivo (Kuijpers & Wilberts 1976 Paloheimo & Thalmann 1977 Kusakari et al 1978) Direct inhibition of the Na K activated ATPase is consequently unlikely to be a major cause of the drug-induced derangements By contrast the dose–response curves of the inhibition of strial adenylate cyclase in vitro and the decrease in the endocochlear potential in vivo are approximately the same (Ahlström et al 1975 Paloheimo & Thalmann 1977) The presumed inhibition of the active transport processes may well be secondary therefore to depression of adenylate cyclase activity and an indirect effect upon the ATPase cannot be excluded In addition there appear to be coincidental alterations in the permeability of the endolymphatic membranes but the evidence has not been sufficient for their precise nature to be determined (Bosher et al. 1973 Melichar & Syka 1978).

The aim of the present investigation was to provide further information about the pathogenesis of ethacrynic acid ototoxicity by continuously following the endolymphatic changes produced in the cochlea by a single intravenous injection of the drug using conventional and ion sensitive microelectrodes Sellick & Johnstone (1974) and Melichar & Syka (1978) have undertaken limited studies of this type upon respectively the endolymphatic Na^+ or K^+ concentration alone In the absence of simultaneous measurements the interpretation of their findings has been greatly hampered so that the increase in our understanding of the underlying mechanisms has been disappointing A deliberate effort was made accordingly to obtain such measurements in the experiments to be described The results provided quantitative measurements of the three effects of ethacrynic acid which were found namely inhibition of the strial transport enzymes decrease in membrane permeability and late depression of oxidative metabolism

METHODS

Animal preparation

Caesarean-originated barrier sustained strains (Charles River) of Sprague Dawley albino rats (150–200 g) and Hartley albino guinea pigs (200–300 g) were used to ensure that the animals ears had not been subjected to the effects of either infection or antibiotics Details of the anaesthesia and animal preparation will be found in the account of a previous investigation (Bosher 1979)

In brief two electrodes were routinely inserted into the cochlear duct through a small opening made over the stria vascularis of the left middle turn in the rat and left basal turn in the guinea pig Surgical anaesthesia was maintained either with sodium pentobarbitone (rat) or with neuroleptanaesthesia (guinea pig) muscle relaxants were not used It was necessary to keep the animals temperature constant (to $<\pm 0\ 4$°C) by means of a thermistor-controlled heating blanket because the ion-sensitive microelectrode readings were temperature sensitive 35°C was chosen for the rat since higher temperatures caused hypernoea and 37°C for the guinea pig. Ethacrynic acid was injected as sodium ethacrynate solution (Edecrin Injection Merck, Sharp & Dohme) into the previously-exposed right femoral vein the injection time being 60 sec Terminal anoxia was produced after varying intervals by the injection of 2 ml air through an indwelling catheter in the left femoral vein without disturbing the electrical screening around the preparation

Types of electrode

A full description of the manufacture calibration and use of the different microelectrodes is again available in the account of a previous investigation (Bosher 1979) One electrode was double-barrelled (tip diameter 2 µm) one channel being a K^+ sensitive electrode made from potassium liquid ion-exchanger (Corning 477317) and the other being a conventional electrode filled with 150 mM KCl for measuring the endocochlear potential Such a double barrelled K^+ sensitive microelectrode was essential to exclude artefacts arising from the spatial separation of the tips of single electrodes Longitudinal variations in the endocochlear potential exist and a 1 mV difference between single electrodes would be equivalent to a K^+ concentration change of 5 6 mM at the endolymph concentration The use of 150 mM KCl as a filling solution prevented the occurrence of an electrode tip junction potential in endolymph which would also have had a similar effect

The other electrode was a Na sensitive microelectrode of the recessed-tip type (tip diameter 5 µm) constructed with a 200 µm-long cone of NAS 11–18 glass (Microelectrodes Inc) These electrodes recorded dc potential changes accurately and gave linear responses in endolymph characteristics which previous workers had failed to achieve with their relatively short cones of Na sensitive

glass in inverted-tip type electrodes Because a 1 mV error in the reading is only equivalent to a Na concentration change of 0 04 mM at the endolymph concentration spatial separation of the tips of the electrodes was not a serious problem In a subsidiary series of experiments, single pH microelectrodes of the recessed-tip type (tip diameter ?.5 μm) made from H -sensitive glass (Corning 0150) were used in place of the Na -sensitive electrodes

Repeated measurements of the calibrating solutions always gave identical readings and a better estimate of the sensitivity of the methods was therefore required. All potentials were recorded to $\pm 50\ \mu$V For the Na sensitive microelectrode this was equivalent to ± 0.002 mM at the 1 mM level and ± 0 0? mM at the 10 mM level The corresponding figures for K were ± 0 28 mM at 150 mM and for pH ± 0 001 unit at pH 7 4 The results are routinely expressed in the form mean $\pm$standard error of mean number of experiments (unless this is given in the adjacent text) The values give ionic concentrations and not activities to enable comparison with other findings to be made more easily

Permeability and flux calculations

Due to the absence of a suitable direct method permeability alterations were quantified by means of the principle introduced by Hodgkin & Katz (1949) and developed for the cochlea by Johnstone and his colleagues (Johnstone 1970 Sellick & Bock 1974 Sellick & Johnstone 1975) In essence the rate of change in the endolymphatic concentration of the ion concerned for any selected moment during terminal anoxia, normalised with respect to the relevant electrochemical gradient is used to calculate the conductance of the ion at that moment according to the equation

$$kG = \frac{\Delta I}{\frac{RT}{zF}\ln\frac{a^e}{a^p}+\Delta E}$$

where R is the gas constant, T is the absolute temperature is the valency of the ion F is Faraday s constant a^e and a^p are the activities of the ion in endolymph and perilymph respectively ΔE is the electrical potential difference between endolymph and perilymph ΔI is the rate of change in the endolymphatic concentration of the ion G is the true conductance and k is a constant Although k is specific for any particular animal species its value is not known precisely and the term kG_i is taken as an index of the permeability of the ion which enables relative permeabilities and permeability variations to be determined accurately In anoxia its value remains unchanged for 3–6 min after the minimum peak in the endocochlear potential following which substantial increases in cation permeability occur The cationic conductances during this short stable period have been shown to be representative of the pre anoxic conditions (Bosher 1979)

When multiplied by the appropriate ionic driving force conductances determined in this way provide an estimate of the rate of concentration change due to the passive forces at any time during the experiment. Thus the contribution of the active transport processes can be derived from the net concentration change according to the equations

$$cJ_{pass} = kG_i\left(RT\ln\frac{a^e}{a^p}+\frac{EP}{F}\right)$$

$$cJ_{active} = cJ_{net} - cJ_{pass}$$

where J_{active}, J_{net} and J_{pass} are the active net and passive ionic fluxes at time t c is the volume area constant for the endolymph system of the animal (so cJ is the rate of concentration change) and EP is the endocochlear potential While the true fluxes (J) cannot be specified because c has not been measured with sufficient reliability the results do enable the various ionic fluxes to be compared with each other and the changes in the fluxes with time can be followed

Table I *Normal endolymph composition*

	Range	Mean S E.M	No of experiments
Rat			
Endocochlear potential (mV)	79.2 –97 0	87 1 ±1 0	35
Endolymph			
[Na] (mM)	0 45 –1.30	0 76 ±0 05	20
[K] (mM)	137 4–167 1	152.2±2.6	15
pH	7 430–7 440	7 436±0 001	5
Guinea pig			
Endocochlear potential (mV)	81 1 –89.5	83 6 ± 0	4
Endolymph			
[Na] (mM)	0 79 –1 36	1 1 ±0 12	4
[K] (mM)	165 8–168 6	167.2±1 4	4

The guinea pig results all lie in the upper portion of the normal range found in a similar but more extensive previous study (Bosher 1979)

RESULTS

Control findings

The values of the endocochlear potential the endolymphatic Na^+ and K concentrations and the endolymphatic pH found during the preliminary control periods are given in Table I They do not differ from the normal results obtained previously in this laboratory (Bosher 1979) and are in good accord with the levels now generally accepted The pH figures confirm the original report of Misrahy et al (1958) Owing to the technical difficulties involved simultaneous perilymphatic measurements were not made However these are not significantly altered by the administration of ethacrynic acid (Bosher et al 1973 Melichar & Syka 1978) and the values determined separately in normal animals (Bosher 1979) were used in the calculations For Na these were 131 7 mM in the rat and 151 7 mM in the guinea pig for K^+ they were 6 8 mM and 3 8 mM respectively

Endolymphatic changes after ethacrynic acid (rat)

A dose of 60 mg kg^{-1} ethacrynic acid was given intravenously because it is the smallest which produces complete abolition of the positive component of the endocochlear potential in the rat (Bosher et al 1973) Potential recordings were obtained in 35 animals, Na results were satisfactory in 20 of these and K^+ results in 15 (combined results were available for 9)

The endocochlear potential (Fig 1) began to decrease after a latent period of 2.4(±0.3) min Its rate of fall was slower and much more variable than in anoxia the greatest negative potential being attained between 9 and 39 min The magnitude of this negative potential was time-dependent values before 15 min were of the same order as those in anoxia but they declined by about half during the next 10 min and then remained at the same level (Table II) Recovery began after 1 to 7 min Once

Table II *The time-dependence of the minimum endocochlear potential after ethacrynic acid (60 mg kg^{-1} i v) in the rat*

Min after injection	<16	16–20	1 –5	>25
Minimum endocochlear potential (mV)				
Mean	−39 1	−35 1	−26 7	−21 9
S E M	± 1 7	± 1 7	± 3.5	± 3.5
Number of animals	12	12	6	5

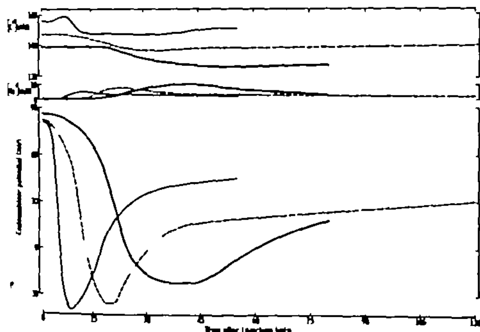


Fig 1 Representative experiments showing the variation in the endolymphatic changes produced by an intravenous injection of 60 mg kg⁻ ethacrynic acid in the rat. The magnitude of the negative peak in the endocochlear potential decreased as the time of its occurrence after the injection increased (Table II) The maximum elevation of the endolymphatic Na concentration was always found a few minutes after the peak in the potential When the potential began to decline the Na concentration began to rise although this is not evident because of the scale used The different patterns of the effects upon the endolymphatic K concentration are illustrated. The experiments were terminated after progressively longer intervals by the induction of anoxia (alterations not shown)

established it was rapid and approximately linear for 6 1(±1 0) min when the rate progressively diminished to a slow constant value only 2–8% of that during the initial phase As a result the endocochlear potential was still 47.0–60 7 mV below the individual control levels at 120 min ($n=3$)

The endolymphatic Na concentration began to rise at the same time as the endocochlear potential began to decrease although this is not evident in Fig. 1 because of its small scale After the negative peak in the potential the rate of increase in the concentration rapidly slowed and then the concentration commenced to decrease so that a maximum of 8 15 (±0 51) mM was produced just after the minimum in the potential The rate of the return of the Na concentration towards normal progressively [illegible] with time and the concentration was 0 72–1 65 mM above the initial levels at 120 min ($n=3$)

The alterations in the endolymphatic K concentration were less consistent in nature In 14 animals there was an overall decrease in the concentration but in 5 of these the decrease was preceded by a transient increase of 1.2–3 4 mM maximal at 6 or 7 min and in the remainder the onset of the decline lagged behind the other changes by up to 5 min (Fig. 1) The total fall in concentration was 5 6–12.9 mM and subsequently the concentration rose again so slowly that the levels at 120 min were still 6 9–9.3 mM below the control values ($n=3$) In the remaining animal there was no variation in the K concentration during the 14 min it was followed

The effects upon the endolymphatic pH were observed in 5 rats Immediately after the

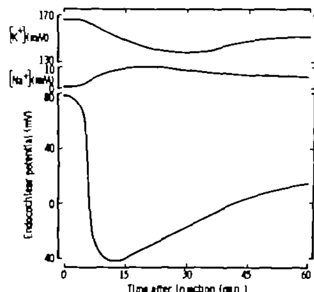


Fig 2 The typical effects produced by intravenous ethacrynic acid (40 mg kg) in the guinea pig The latent period was 1 min and the positive component of the endocochlear potential was completely abolished for 1 min Although not apparent at this scale the endolymphatic Na concentration began to rise when the potential commenced to decline The endolymphatic K concentration remained unchanged for 3 min The differences in scale from Fig 1 will be noted

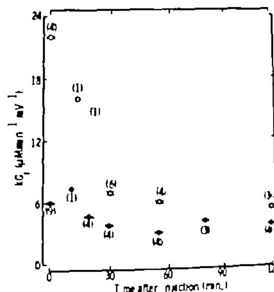


Fig 3 The action of intravenous ethacrynic acid (40 m kg) upon the gross permeability of the endolymphat membranes in the rat calculated as described in the tex For the purposes of determining the effect of the dru upon active transport the decrease in both the Na (and the K (O) permeabilities was considered to be pr gressive The significance of the solitary Na finding 11 min was uncertain because of the technical difficult experienced at this time However the active transpo results subsequently suggested that a transient increa in Na permeability did occur Each point indicates mean and S E.M for the number of experiments sp cified in parentheses The zero time values are the norm levels taken from Bosher 1979

injection there were differing and transient alterations in three experiments but no change in the remaining two Thereafter the pH remained constant until the experiments were terminated at 100 min The interpretation of the early changes is difficult The ethacrynic acid administration often resulted in short lived alterations in respiration (combinations of stimulation depression and irregularity) and its influence upon the blood pH is conjectural However there was no consistent pattern about the initial action upon the endolymphatic pH and the conclusion that it was not directly affected by ethacrynic acid seems reasonable in view of the subsequent invariant level

Endolymphatic changes after ethacrynic acid (guinea pig)

The effects of ethacrynic acid were followed in 4 guinea pigs for 60 min to determine whether any significant differences from the rat occurred since the results of perilymphatic perfusion with ethacrynic acid were not the same in the two species (Bosher 1979) Preliminary experiments revealed that the smallest intravenous dose of the drug which completely abolished the positive component of the endocochlear potential (i e which was equivalent to the dose given in the rat) was 40 mg kg^{-1} and this was the dose administered. After a latent period of 1 min the endocochlear potential fell to a minimum of $-39.5(\pm 2.8)$ mV at 12 8($\pm$1 4) min a value which is not significantly different from the anoxic values as would be expected from the time relationship Recovery of the potential commenced after 1 to 2 min and was biphasic in character The endolymphatic Na concentration increased by 10 7($\pm$1 2) mM and the K concentration decreased by 34 1($\pm$5 1) mM before both began returning towards normal Compared with the rat recovery was somewhat

...er in the case of Na but faster in the case of K. As described below these findings (Fig. 2) indicate that ethacrynic acid given intravenously has essentially the same actions in the two species, the differences found being only of a minor quantitative nature.

Permeability alterations

Each experiment was terminated with anoxia induced at varying times after the initial injection and the relative alterations in the permeabilities of the endolymphatic membranes which had arisen were calculated as described earlier. The results (Fig. 3) showed that the ethacrynic acid had produced decreases of 40(±4)% in the Na permeability ($n = 10$) and 78(±3)% in the K permeability ($n = 9$) in the rat. These permeability effects were present at 30 min and were long-lasting, no evidence of recovery being present at 120 min. Attempts to determine the time course of the reductions more precisely were unsuccessful. This was because some ethacrynic acid probably remained in the inner ear tissues when anoxia was induced shortly after the drug's administration. In these circumstances the permeability changes would eventually develop fully during the anoxic period due to its continued action. It will be noted that these permeability effects proved to be different in type to those expected from our preliminary investigation (Bosher et al. 1973). An advantage of the dose of ethacrynic acid selected was that the rise in the endolymphatic Na concentration was well below the level of 12 mM. This is the concentration which is associated with a marked increase in the permeability of the endolymphatic membranes to cations (Bosher 1979). The complications of a secondary change of this type were therefore completely avoided. The guinea pig experiments were too few for the total range of the effects to be determined accurately. But the mean reductions found in the Na and K permeabilities were 45% and 68% respectively (i.e. about the same as in the rat).

Effect on active transport

The changes in the endolymphatic ion concentrations provide very little information by themselves about the effect of ethacrynic acid upon the active transport mechanisms. The marked alterations in the endocochlear potential produce equally marked alterations in the passive fluxes and these must be taken into account first. However, this can be done as described earlier and the action upon the ion-transporting mechanisms is then revealed.

In every animal the active processes responsible for Na and K transport and endocochlear potential production were completely abolished by the time of the minimum peak in the potential. A problem arises about the exact interpretation of the details of this action. As explained above, it was not possible to determine experimentally the precise time courses at the beginning of the permeability effects. So the initial time courses illustrated in Fig. 3 were used in the calculations. Although these were undoubtedly good first approximations, the early differences between the various processes apparent in Fig. 4 might have been due to small variations in the true time courses of the permeability effects rather than actual divergences between the processes themselves. For example, the initial small rise in Na activation might have been due to a slight transient increase in Na permeability before it declined to its later level (Fig. 3). Whatever their cause, these initial differences were relatively minor in nature and activation was reduced to zero at the time of the minimum endocochlear potential in every case. Consequently, the principle effect of the ethacrynic acid in this respect was to fully inhibit all the transport processes measured at about the same overall rate.

At and after the time of complete inhibition the reductions in permeability measured individually could be used in each experiment. Thus, similar problems do not arise about the findings in this period. These revealed that the Na transport increased much more quick-

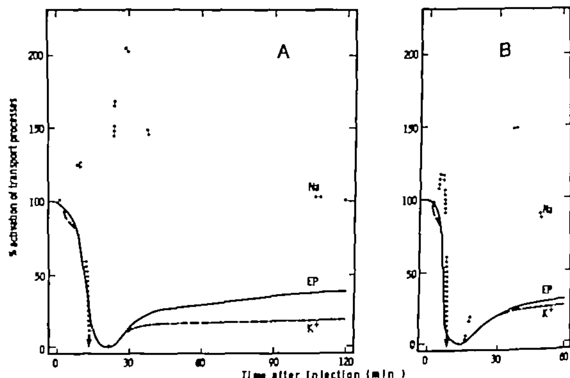


Fig 4 The action of intravenous ethacrynic acid upon the active transport processes responsible for maintaining the endolymphatic composition in the rat (A) and guinea pig (B) calculated as described in the text. The representative experiments illustrated are the one picted in Fig. 1 (170 min animal) and Fig. – The port rates have been normalised with respect to the during the control period (100 %)

ly and to a much higher level of activation than the K^+ transport or the potential production during the early part of the recovery period Then at 26(±2) min in the rat there was a sudden and substantial decrease in the rate of Na^+ transport which had reached an activation level of 241(±19)% At the same time the obvious later decline in the rates of K transport and potential production commenced after their previously progressive increases to activation levels of 18(±3)% and 20(±3)% respectively This is not clearly visible in Fig 4 because of its small scale Thereafter the increase in the rates of recovery of the K transport and the potential production were very slow This is the reason why complete functional recovery after a single intravenous dose of ethacrynic acid takes up to 10 hours (Bosher et al 1976 Brummett et al 1977)

It must be stressed that the results represent alterations in the activation levels of the processes concerned The average ratio of the amount of K^+ transported to the amou Na^+ transported during the control pe was 13 1 in the rat At the time of the pe the Na^+ activation values this ratio had fa to 1 1 but had increased to 2.5 1 at 170 min Thus the amount of K^+ transported always exceeded the amount of Na except for the brief period when they were equal The findings in the guinea pig studies demonstrated that the effects of intravenous ethacrynic acid were qualitatively the same as in the rat although quantitative differences were present (Fig 4) Detailed presentation of these differences does not seem justified because of the small number of experiments performed. But for example the ratio between active K^+ and Na^+ transport in the control period was 8 1 compared with 13 1 and the sudden decrease in the recovery rates occurred at 35 min compared with 26 min

From the foregoing description it follows that the unchanged levels of the endolymphatic K concentration which have been de-

scribed previously in the guinea pig and cat (Brusilow & Gordes 1973 Silverstein & Yales 1971) were due to an equivalent effect upon both the passive and the active fluxes This was certainly the case in the one rat exhibiting this phenomenon in the present investigation where the inhibition of the transport processes followed its usual course and the reduction in the passive K flux precisely paralleled the reduction in the active K transport.

DISCUSSION

The nature of the ototoxic action of ethacrynic acid

The present investigation has confirmed that ethacrynic acid has three main toxic effects upon the endolymph system and it has been possible to obtain measurements of their respective magnitudes One of the effects is inhibition of the strial transport mechanisms concerned with endolymphatic homeostasis The underlying enzymic processes seem to have much the same sensitivity to ethacrynic acid in the initial stages and this would be expected if the primary action of the drug was upon a regulatory adenylate cyclase as suggested by Paloheimo & Thalmann (1977) However the marked differences in ion transport which developed during the recovery phase are less easy to explain on this basis A direct action upon the transporting enzymes therefore remains a distinct possibility and the results do not help in distinguishing between the two alternative concepts

One feature which has been confirmed is the existence of a relatively long latent period before any change occurs in the active transport. The latent period after an equivalent dose of furosemide administered by the same route is less than one minute (Bosher unpublished observations) so the method of administration and access to the strial cells do not appear to be important factors Delay due to chemical transformation of the drug, say into a thiol adduct, has been proposed but seems to be excluded by the findings of Fox & Brummett (1974*a* *b*) In consequence the nature of the molecular interactions responsible for the latent period remains unknown

The second major effect which has been documented is the decrease in the overall permeability of the endolymphatic membranes This pursues a quite different time course from the enzyme inhibition developing over 30 min and showing no signs of recovery at 120 min Similar reductions in ionic permeability occur in the kidney (Burg & Green 1973) but the maximum decrease there has only been by about 70% i e considerably less than in the ear Another unusual aspect of the endolymphatic changes is the preferential diminution in the K permeability The situation accordingly suggests that ethacrynic acid might act upon some specialised region of the boundary membranes Nevertheless more direct evidence of this is lacking Whatever their site the decreases in permeability will tend to reduce the magnitude of the passive cation movements Thus the recovering transport mechanisms will be able to produce larger increases in the endolymphatic concentrations than if these passive movements were normal The concentration alterations alone therefore greatly overestimate the degree of recovery of the transport processes

The last of the principal effects delineated is the substantial reduction in the rate of recovery starting at about 26 min in the rat and a few minutes later in the guinea pig At this time the cation concentrations have returned less than halfway toward the control values and their rates of change are comparatively slow The diminution in the active transport, consequently is most unlikely to be the result merely of a reduction in enzyme activation secondary to the alterations in the ionic concentrations particularly in view of the severity and rapidity of the fall in the Na transport. A well-known action of ethacrynic acid is its depression of oxidative metabolism and ATP production which often does not become

manifest in other tissues for some time after its administration (Rossum & Ernst 1978) It therefore seems reasonable to propose that a delayed decrease in strial ATP production is responsible for the delayed decrease in active strial transport the cause of the excessively prolonged recovery described previously (Bosher et al 1976 Brummett et al 1977) The possibility of the initial inhibition of active transport being due to diminution in the strial energy supply is completely excluded by the results of Kusakari et al (1978) An increase in arterial oxygen tension has been reported to protect the cochlea partially against the toxic effects of ethacrynic acid but whether this is brought about by some action on cochlear oxidative metabolism has yet to be determined (Prazma & Pecorak 1975)

The present investigation has been concerned with the acute functional changes in the endolymph system after a single intravenous dose of the drug It has also provided a unique opportunity to correlate these with the morphological alterations in the stria vascularis Systematic ultrastructural examination of typical animals at frequent intervals was therefore undertaken and the results will be presented in a subsequent paper Other problems concerning the ototoxic action of ethacrynic acid remain to be resolved such as the consequences of the new pathological features which appear in the stria vascularis after 12 hours or more (Bosher et al 1976 Brummett et al 1977) the aetiology of the disastrous cumulative effects of small daily doses (Crifò 1973) and the basis of the potentiation by aminoglycoside antibiotics specific to the inner ear (Fox & Brummett 1979)

The normal processes controlling the endolymphatic composition

The endocochlear potential is now generally believed to consist of two main components The first is a positive electrogenic potential usually attributed to active K transport although other possibilites could exist (e g proton transport) Direct experimental evidence is scanty and consists essentially of the discovery of a correlation between the level of the endocochlear potential and the strial K transport when the endolymphatic K concentration is decreased by perfusion (Sellick & Bock 1974) In the studies reported here a similar correlation has now been found in a second situation namely after the administration of ethacrynic acid As described above a reasonably close correspondence between the activation levels of the potential producing and K^+ transporting processes occurred throughout the recovery period

It is true that a consistent small discrepancy was present the potential producing processes always appearing slightly more active than the K^+ transporting ones There are two possible explanations for this Firstly ethacrynic acid is known to increase the trans-epithelial electrical resistance (e g Burg & Green 1973) so the transport mechanisms could produce a higher potential for any given level of activation than in normal circumstances The consequent overestimation of the recovery of these processes would tend to result then in the discrepancy under consideration Alternatively the discrepancy might indicate the existence of another subsidiary source for the positive electrogenic potential At present, it is impossible to determine which possibility is correct in the absence of satisfactory direct methods of investigation Nevertheless the discrepancy is only a small one and there seems no doubt that active K transport is the major source of the positive component of the endocochlear potential

The other principal component is the negative potential found after the positive component is abolished by anoxia or inhibitors has been established that this negative component behaves quantitatively as a modified K diffusion potential which is dependent upon the measured selective permeability of the endolymphatic system to K and upon the K and Na concentration gradients between endolymph and perilymph apart from a minor contribution due to the remaining ions (Bosher

979) However the location and extent of e biophysical component of the endolymph-ic membranes responsible for the overall lective permeability has yet to be deter ined A possible third component of the en-docochlear potential has been described in the inea pig (Sellick & Johnstone 1974 Kusa-ri & Thalmann 1976 Kusakari et al 1978) it has been shown to be completely absent the rat (Bosher 1979) Consequently it oes not complicate the interpretation of the xperiments on this species reported here

In the 9 rat experiments in which simultane us Na and K results were obtained the ean diffusion potential calculated from the tandard Hodgkin & Katz (1949) equation and he measured values at the time of the mini-mum potential peak was −20 0(±4 4) mV The verage endocochlear potential actually found was −24 7(±3 9) mV the discrepancy of 17(±0 9) mV being identical with the one described in other situations (Bosher 1979) The difference in the value of this negative peak to the one found after anoxia in normal animals is due to the decrease in the relative permeability of the endolymph system to K after ethacrynic acid the measured Na K permeability ratio at this time being 0 63(±0 08) compared with the normal 0.27 in the rat

Finally the results also provide evidence about the relationship between the mechanisms responsible for cation transport As reported, the normal ratio of active K to Na transport is 13 1 in the rat and 8 1 in the guinea pig. Although the latter value is less than the 66 1 derived theoretically for the guinea pig by Sellick & Johnstone (1975) it is still well outside the coupling ratios of the Na K activated ATPases In addition the large differences in the active transport systems demonstrated during the recovery period indicate that most of the K transport must be independent of the Na transport. These findings consequently provide further experimental evidence in support of the view that some enzyme other than a Na K -activated ATPase is directly responsible for the potential generation and at least most of the K transport (Kuijpers & Bonting 1970*b* Sellick & Bock 1974 Sellick & Johnstone 1975 Paloheimo & Thalmann 1977 Kusakari et al 1978) It follows from this that the K transport will be largely and possibly completely electrogenic in nature and this is confirmed by the close association between the K transporting and potential-producing systems described in the recovery period

The importance of Na K -activated ATPase in the endolymph system has not been in question since the initial studies of the effects of ouabain (Konishi & Mendelsohn 1970 Kuijpers & Bonting 1970*a*) Moreover it is present in the stria vascularis in a high concentration (Kuijpers 1974 Kuijpers & Bonting, 1969 Matschinsky & Thalmann 1970) but it cannot be concerned directly with endolymphatic cation transport. Instead preliminary results in this laboratory (Bosher unpublished observations) suggest its role to be an indirect one such as maintaining the internal ionic environment of the transporting cells

ACKNOWLEDGEMENT

I am grateful to the Department of Medical Illustration The Middlesex Hospital for preparing the diagrams

ZUSAMMENFASSUNG

Endolymphatische Veränderungen infolge intravenöser Injektion von Ethacrynsäure an Ratten, 60 mg kg⁻¹ wurden bis zu 120 Minuten lang beobachtet unter Verwendung konventioneller und ionenssensitiver (Na K und pH) Mikroelektroden Man fand als Ursache 3 deutliche Wirkungen auf das Endolymphsystem. Zunächst wurden die strialen potential-produzierenden und kationentransportierenden Prozesse total gestoppt. Wiederherstellung begann schnell und war zunächst rapide. Dann verlangsamte sie sich erheblich, vermutlich infolge der Abnahme der strialen Energieproduktion mit langsamem Beginn und langer Dauer Gleichzeitig mit diesen Wirkungen auf die aktive Transportation trat eine Veränderung der gesamten Kationenpermeabilität des Endolymphsystems auf Dies hatte einen verschiedenartigen Zeitablauf und betraf K erheblich mehr als Na Die Befunde gaben außerdem weitere Informationen über den Mechanismus, der für die normale Beschaffenheit

der Endolymphe verantwortlich ist. Qualitativ ähnliche Resultate wurden bei Experimenten mit Meerschweinchen gefunden.

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S K Bosher
Ferens Institute of Otolaryngology
The Middlesex Hospital Annex
Cleveland Street
London WIP 5FD England

Acta Otolaryngol 89: 419–423 1980

SPEECH DISCRIMINATION OF PATIENTS WITH HIGH FREQUENCY HEARING LOSS

H Kiukaanniemi

From the Department of Otolaryngology University of Oulu Oulu Finland

(Received March 7 1979)

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…linical experience has often given the impres-…on that some patients with relative serious …w-pass hearing loss can utilize the remnants …f their hearing better than some with moder…te but in other respects similar hearing loss. …n an earlier experiment made to examine this …henomenon three degrees of low-pass hear…ng loss were simulated using a filter apparatus …nd the corresponding discrimination curves …neasured using young subjects with normal …earing (Kiukaanniemi 1979*a*) It was shown …hat theoretically there really are some sig…nificantly disadvantageous high frequency …earing losses which may yield markedly poorer discrimination scores at high sound pressure levels than other more serious hear…ing losses of the same type

One case of this kind proved to be a linearly decreasing hearing loss of 22 dB/octave beginning at about 250 Hz. The discrimination curve for this hearing loss crossed the curve yielded by a parallel hearing loss beginning at 125 Hz. The crossover point was 84 dB re 2×10^{-5} N/m^2 and 75% discrimination This phenomenon has not been reported before in the literature and it encouraged the author to attempt a measurement of the discrimination curves of patients having an equivalent clinical high frequency hearing loss A further indication for this study was the lack of earlier results concerning the discrimination ability of Finnish-speaking patients with various grades of high frequency hearing loss.

MATERIAL AND METHODS

72 patients whose pure-tone audiogram showed typical high frequency hearing loss were selected for further examination An anamnesis was taken in order to outline 1) etiology of the hearing loss 2) use of a hearing aid or other auxiliary facilities 3) the patient's subjective opinion on his/her difficulties in daily life

The ear status and pure-tone threshold of the patients were examined and those showing recruitment in Reger's test were excluded so as to achieve a loudness function and its influence on the threshold curve equal to the preceding experimental study The elimination of recruiting ears may increase the relative number of retrocochlear losses in the present material The preceding experimental study with filtered words using normal-hearing subjects gave a reason to anticipate a very disadvantageous high frequency hearing loss among non-recruiting ears Discharging ears were similarly eliminated from the subsequent tests

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S K Boshe
Ferens Institute of Otolar ngology
Th Middlesex Hospital Annexe
Cle eland Street
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MATERIAL AND METHODS

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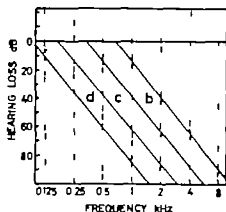


Fig 1 The pure-tone audiograms for the patients tested in groups B C and D were located in the areas marked *b c* and *d* respectively The slope of the audiograms is 2↓ dB/octave

Table II *Discrimination scores for groups C and D at six sound pressure levels and significance of the differences in the Student's t-test*

Output level (dB) SPL	Group D Discr % ± S D	Group C Discr % ± S P	Significance in Student's t-test
57	2.0±1 6	17 1±4.3	$p<0 001$
67	16.2±4 0	37.3±7 0	$p<0 001$
77	29 9±6.2	47 0±8.1	$p<0 001$
87	46 8±7 0	51.3±8.4	$p<0 1$
97	56.5±6.5	50 ±7 8	$p<0 01$
107	63 0±7 5	47.5±8.2	$p<0 001$

Ten cases of occupational hearing loss were excluded from the subsequent tests because of showing recruitment in Reger s test Two cases were excluded because of acute dis charging

The aetiological factors involved in the 60 cases studied here are given in Table I Im pulse noise (pressure hammer rifle shots etc) was the main cause (63%) amongst the pa tients having occupational high frequency hearing loss selected here only one third of them (38%) using ear plugs regularly All the patients used a hearing aid 66% controlled the output levels themselves but 30% of these adjusted the output level or compressor con- trol wrongly in the opinion of an experienced hearing aid acoustician When the patients were asked to select a hearing aid from an assortment of 15 different devices in a session guided by the same hearing-aid acoustician, 94% of them subjectively chose one with high frequency emphasis the amplification begin- ning at 300–500 Hz.

52% of the total group of 60 patients claimed that group conversation caused them most inconvenience while the rest suffered most inconvenience when using the telephone (26%) or because of the subjective distortion (breaking up) of sounds (22%)

The preliminary conversation screening and examination reduced the first group of 72 patients to 60 who were then divided into three groups of 20 according to their degree of hearing loss in a pure tone audiogram These groups B C and D occupy the areas marked *b c* and *d* respectively in the pure tone au-

Table I *Aetiological factors in high tone hearing loss found in the present study*

	Number of cases	%
Acoustic trauma, occupational hearing loss	25	42
Perinatal factors (asphyxia, maternal toxemia)	20	33
Ear infections later in life	7	12
Viral infections	5	8
Bacterial meningitis		3
Uncertain vascular etiology	1	2
Total	60	100

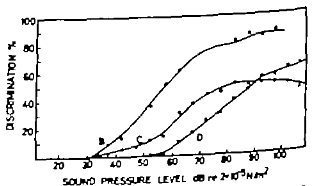


Fig 2 Mean discrimination curves for three groups (B C D) with high-tone hearing los corresponding to audiograms within the areas *b* and *d* in Fig 1

able III *The effect of presbyacusis on discrimination of low-pass filtered speech in age groups (40.1 (I) and 50.3 (II) years*

	Filtration d		Filtration	
Input level dB SPL	Group I Discr % ± S.D	Group II	Group I Discr % ± S.D	Group II
[illegible]	10.1±5	9.8 5 0*	45 0±6 9	43.0±6 0*
[illegible]	52.3±6..	51.0±5 0*	63.0±6 1	63..±5.9*
[illegible]	79 1±7.3	77 0±6.9*	75 1±7.8	73 9±6 6
[illegible]	85.6+8	84 0±8 0*	77 0±7.8	75 4_7 1

p>0 05 Student' -test.

iograms in Fig. 1 The mean ages for the roups were 54 9 (B) 51.3 (C) and 42 0 (D) ears. Each patient was guided to a soundroof room where 10 lists of 25 recorded PB words (Palva 195? Jauhiainen 1974) were resented to him monaurally with masking of he opposite ear where needed The earphones sed were MX 41/AR TDH 39 and the tape ecorder a Revox A77 The sound pressure evel being monitored using a Madsen OB 60 udiometer The words were presented first at level 40 dB above the speech reception hreshold (SRT) of the patient established oughly by the method described by Palva 1952) The next list was then presented at a evel 5 dB lower and so on until the discrimiation score was 10% or less. The background noise was lower than 45 dB (A) The patient repeated the words and was encouraged to guess if he was not sure about the given word he erroneous answers being written down for further investigation. The average discrimination scores for each test group were calculated n 5 dB steps from 3? to 11 dB re 2×10^{-5}N/m²

RESULTS

The average discrimination curves for the groups B C and D are seen in Fig. 2, in which the difference between the scores of groups C and D at very high and rather low sound pressure levels are statistically significant (Student's t-test $p \leq 0$ 001) as seen in Table II

DISCUSSION

The present findings corroborate those of the simulation experiments carried out on subjects with normal hearing (Kiukaanniemi 1979*a*) that is there also seems to exist a real clinical group of patients (C) who have a weaker discrimination ability than any other group (D) with a poorer pure-tone audiogram The pure tone threshold of the group C begins to decrease linearly by 22 dB/octave at the frequency range 200–300 Hz, and the discrimination curves of C and D cross at the point 90 dB re 2×10^{-5}N/m² and 51% discrimination the curve for group C failing to rise significantly above this level Since the ears showing recruitment in Reger s test were excluded the distortion associated with cochlear signal processing was probably small

The difference in the mean ages between the groups C and D was relatively large 9 3 years Palva & Jokinen (1970) found using the monaural and Matzker's binaural test that speech discrimination scores in the filtered speech test began to fall by the fourth decade and that there was marked asymmetry in the age groups over 60 years. Using masking noise (S/N ratio +22 +1 +? and −3 dB) Jokinen (1973) also demonstrated an almost significant difference (p<0 05) in discrimination scores between the age groups 40–49 and 20–29 years but not between the groups 40–49 and 50–59 years The filtered speech test may be more sensitive in revealing discrimination loss caused by presbyacusis (Jokinen, 1973)

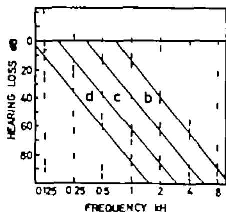


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87	46 8±7 0	51.3±8.4	$p<0\ 1$
97	56.5±6.5	50.–±7.8	$p<0\ 01$
107	63 0±7.5	47.5±8.–	$p<0\ 001$

Table I *Aetiological factors in high tone hearing loss found in the present study*

	Number of cases	%
Acoust c trauma occupational hearing loss	25	42
Perinatal factors (asphyxia, maternal toxemia)	?0	33
Ear infections later in life	7	12
Viral infections	5	8
Bacterial meningitis		3
Uncertain vascular etiology	1	
Total	60	100

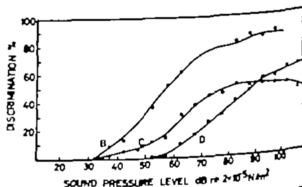


Fig 2 Mean discrimination curves for three groups (B, C D) with high-tone hearing loss corresponding to audiograms within the areas *b* and *d* in Fig 1

...mpared with that of the English speaker ...lthough there are large individual differences ...speech spectra (Byrne 1977) the linguistic ... of the Finnish language may also add ...the errors caused by upward masking of the ...ment F in group C. Conversely however ...also appears from the present results to be ...ier to utilize the remnants of one's hearing ...one's audiogram belongs to groups B or D ...pward masking probably effectively eliminates the advantage which some Finnish patients (group C) with high frequency hearing loss have due to the fact that the speech power ...their language is concentrated in the frequency area where their residual power of hearing lies

The present results and those of the preceding experimental study suggest that the sloping frequency area where the disadvantageous masking effect is present is rather narrow about ½ octave Nevertheless mild high frequency hearing losses are frequently located precisely in this area. These facts make it important in clinical practice to single out patients belonging to group C when selecting a hearing aid The distorting masking noise of the first formant is best eliminated by a hearing aid in which amplification starts at 500 Hz As seen here the patients in this group subjectively chose such a hearing device because of its significantly better discrimination ability Another possibility would be a device which would present the main frequency areas of F and F to opposite ears Some patients however show a third type of masking, central masking (Danaher & Pickett, 1974) which eliminates even the advantage of this kind of hearing aid Also the exact separation is difficult, because F and F very often overlap

ZUSAMMENFASSUNG

Sprachverständniskurven für drei Gruppen von Patienten mit einem schweren, mittelschweren und leichten Hörverlust wurden mit den finnischen PB-Wörtern gemessen 20 Patienten wurden in jeder Gruppe untersucht. Das Audiogramm jedes Patienten sank parallel 22 dB/Oktave Die Ohren mit dem positiven Rekruitment in Reger' Tests wurden ausgeschlossen. Die Gruppe mit d... ...kriminationswerte auf hasten Lautstarken als die Gruppe mit dem schweren Horverlust — eine Erscheinung, die man nicht früher rapportiert hat. Die Resultate stimmen doch mit dem früheren Experiment überein, wo die äquivalenten sensorineuralen Hörverluste mit einem Filterapparat simuliert wurden. Die Ursachen und die Bedeutung dieser Erscheinung für die Auswahl des Hörapparates wird diskutiert.

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Dr Heikki Kiukaanniemi
...ryngology
...a 22 *Finland*

Therefore two additional groups with mean ages of 50 3 and 40 1 years were retested in order to study the significance of presbyacusis in the circumstances of the present study Fifteen subjects were tested in each group simulating the hearing losses C and D using the filter equipment described earlier (Kiukaanniemi 1979*a*) The results in Table III show that the small differences caused by presbyacusis cannot wholly explain the crossing of the discrimination curves C and D in the present study

The discrimination curve for group B shows a plateau at the level 75–80 dB due to the differential sensitivity to high and low frequencies (Boothroyd 1967) as was also found in the experimental part of this study (Kiukaanniemi 1979*a*) In the present case the plateau is relatively small however and not of any great clinical importance and will thus not be discussed any further in this connection

The crossing of the curves C and D on the other hand is worth further consideration It has not been reported before although its major cause the upward spreading of masking noise has been mentioned by many authors (Jerger et al 1960 Bilger & Hirsh 1956 Rittmanic 1962 Martin & Pickett 1970 Byrne 1977) Martin et al (1972) and Danaher et al (1973) have shown in experiments using synthetic vowels that when F_2 was presented alone there was a marked difference in performance between the normal subjects and those with sensorineural hearing loss A much greater difference was found when the subjects listened to F_2 transitions in the presence of F_1 energy The performance of the subjects with sensorineural hearing loss was much poorer than that of the normal subjects although there was a marked variability The existence of a variable masking effect of F_1 on F_2 discrimination depending on the degree of hearing loss as found in the present study would obviously explain the great variability in the performance of person with sensorineural hearing loss described by Danaher & Pickett (1974) Altogether the poor performance of groups C may be of importance for hearing aid evaluation in the case of such patients The poor performance is mainly a result of three factors 1) the emphasized masking effect of F_1 on F_2 discrimination 2) an unspecific masking effect on low frequency noise on high frequency information and 3) language specific linguistic and phonetic factors.

Danaher & Pickett (1974) have shown that the masking effect of F_1 on F_2 may be of both a forward and backward type and it is even possible in monaural hearing but found no simple correlation between the extent of masking and the configuration of the pure tone audiogram Although they measured syllabel recognition rather than recognition of speech sound information their results parallel the present ones in other respects as far as discrimination is concerned

The recognition errors found in simulation tests concerning various types of hearing loss (Kiukaanniemi & Määttä 1979) suggest that the poor discrimination found in group C is clearly derived from two types of error (*a*) the ratio between errors in vowels and errors in consonants increases markedly as the sound pressure level increases above 97 dB re 2×10^{-5} N/m² (*b*) Errors in the place of articulation remain at a relatively high level for practically all place categories at 97 dB re 2×10^{-5} N/m² On the other hand errors in the manner of articulation reach a lower error level with a steep decrease in errors for obstruents i e consonants with vowel-like formant structure show a rising type of identification score This fact argues in favour of the significance of the masking effect of F_1 on F_2 discrimination in group C

On the other hand the masking effect of F_1 on F_2 is particularly significant in languages like Finnish which are rich in vowels. In an analysis using Finnish and English speakers and phonetically balanced word material (Kiukaanniemi 1979*b*) the speech power of the Finnish speaker was shown to be centred significantly more on the low frequencies



Fig. 1 The macula of the saccule following ultrasonic irradiation towards the vestibule with 100 milliwatts (H&E, 200) es Endolymphatic space n, neuroepithelium om otolithic membrane

1.5% veronal buffered osmium tetroxide (Bredberg et al 1965) The results were compared with that occurring after a 4-week recovery period assessed from conventional histological methods involving Heidenhain's susa fixation nitrocellulose embedding, serial sectioning and staining with haematoxylin and eosin.

RESULTS OF VESTIBULAR IRRADIATION

Vestibular disturbances observed immediately after recovery from the anaesthesia included paralytic nystagmus accompanied by a head-bobbing motion and loss of balance control with the cat falling onto its irradiated side The severity of these effects was dependent on the intensity and duration of irradiation with the strongest reaction following application of 100 milliwatts for 20 min. The direction of irradiation was found to be critical and was dependent on ultrasound being aimed towards the position of the ampulla of the superior semicircular canal. In most cases completely normal locomotory function was regained within ten days following the irradiation. These symptoms of balance dysfunction indicated vestibular irritation and animals not displaying this response were assumed to be incorrectly irradiated and were excluded from subsequent histological examination.

Four weeks after vestibular irradiation histological changes were observed within the vestibular neuroepithelium Due to the variability of cellular changes it was impossible to relate the extent of damage to the ultrasound dosage and the following changes were observed following 100 milliwatts irradiation In the saccule the otolithic membrane was often lifted away from the neuroepithelium (Fig 1) causing damage to the hair cell cilia. The sensory epithelium lacked clarity of cellular detail and intercellular spaces occurred in this layer and in the underlying stroma. The basement membrane separating the epithelial layer from the stroma was not clearly differentiated. The otolithic membrane in the utricular macula was more resistent to damage but cellular disruption and cilia loss within the neuroepithelium was widespread The cell walls were destroyed and the nuclei of the supporting cells and sensory cells clumped together into thin strands resulting in disorganisation of the normal stratified appearance of this epithelial layer (Fig. 2) Cellular detail in the underlying matrix was somewhat obscure

Degenerative changes occurred to a lesser degree within the cristae in the ampullae of the semicircular canals Deformities of the cupula commonly occurred in the superior ampulla, together with loss of cellular detail in the neuroepithelium The extent of these changes was variable but there was a tendency for the utricular side of the crista to be more severely damaged than the semicircular canal side (Fig.

Acta Otolaryngol 89 424–432 1980

THE EFFECT OF ULTRASONIC IRRADIATION ON THE STRUCTURAL INTEGRITY OF THE INNER EAR LABYRINTH

S B Barnett

From the Ultrasonics Institute Sydney Australia

(Received April 26 1979)

Abstract The histological appearance of the inner ear labyrinth was examined following ultrasonic irradiation of the vestibule and the cochlea in cats and guinea pigs Directing ultrasound through the round window towards the ampulla of the superior semicircular canal produced severe balance dysfunction together with histological damage throughout the vestibular labyrinth Cochlear damage was restricted to a small region of the basilar membrane proximal to the round window When ultrasonic energy was directed into the cochlea cellular disruption extended over an area of at least two cochlear turns and included changes in the stria vascularis as well as to hair cells The outer hair cells were found to be more sensitive than the inner hair cells to the damaging effects of ultrasound

The alleviation of the vertiginous symptoms of Meniere s disease by the application of ultrasound into the diseased vestibule is an established practice (Arslan 1964 James 1963 Sjoberg et al 1963) Kossoff et al (1967) developed a technique which avoids the risk of facial nerve trauma and hearing impairment by irradiating the vestibule through the round window The success of this treatment has been confirmed from examination of patient case histories over a four year post-operative period (Barnett & Kossoff 1977) Although there have been several studies on the effects of ultrasound in the inner ear (Brain et al 1960 James et al 1964 Giancarlo et al 1965 Stahle & Sugar 1973 Lundquist et al 1978) the mechanism involved in alleviating the symptoms remains unknown The present study was undertaken to assess the extent of pathological changes in the vestibular and auditory labyrinth following irradiation through the round window and to determine the optimal dosage for vestibular damage

MATERIALS AND METHODS

The round window ultrasonic applicator designed and constructed at the Ultrasonics Institute consists of a flat 3.5 MHz transducer 1 2 mm in diameter which is mounted in a cylindrical holder Ultrasonic energy propagates in a beam diameter of 1 2 mm for a distance of 6 mm and then diverges to reach a diameter of 2 mm at a distance of 10 mm from the transducer Irradiation was applied at average intensities of from 5 W/cm^2 to 10 W/cm^2 for a duration of 20 minutes during which the transducer was fixed in position with a micromanipulator These intensities correspond to 50 milliwatts and 100 milliwatts respectively of acoustic power measured by the radiation pressure technique Coupling was achieved with the irradiation site and the ultrasound transducer submerged in distilled water Following surgical exposure of both tympanic bullae the right inner ear was irradiated while the left ear was sham-irradiated and served as a control

In the first part of the investigation the vestibule of six cats was irradiated through the round window and the effect on cell structure was studied in the cochlea and vestibule In a further series of experiments with six cats and six guinea pigs ultrasound was directed along the longitudinal cochlear axis from either the round window or the helicotrema. Immediate structural changes were determined by examination of surface preparations which involved the removal of the otic capsules under deep anaesthesia and fixation for 90 min with



g. 1. The macula of the saccule following ultrasonic irradiation towards the vestibule with 100 milliwatts (H&E, 250×). es, Endolymphatic space; n, neuroepithelium; om, otolithic membrane.

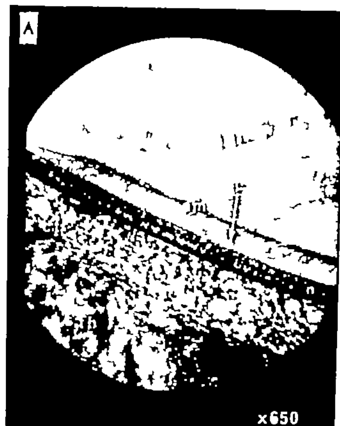
1.5% veronal buffered osmium tetroxide (Bredberg et al. 1965). The results were compared with that occurring after a 4-week recovery period, assessed from conventional histological methods involving Heidenhain's susa fixation, nitrocellulose embedding, serial sectioning, and staining with haematoxylin and eosin.

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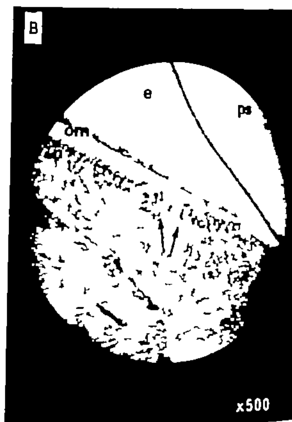


Fig 2 (A) The utricular macula from a control cat ear showing the normal pseudostratified neuroepithelium with sensory cilia projecting into the otolithic membrane (H&E ×650) (B) The utricular macula showing vacuolation clumping of the sensory cells into thin strands and total loss of their cilia following vestibular irradiation with 100 milliwatts (H&E, ×500) *c* Cilia, *ct* connective tissue stroma *p* perilymphatic space *tb* temporal bone.

3) The crista of the lateral semicircular canal was rarely affected while the posterior crista and cupula appeared normal in every animal following irradiation

In the cochlea the cellular components of the organ of Corti were unchanged except for a restricted region proximal to the round window where cytoplasmic vacuolation in the inner and outer sulcus cells and some loss of outer hair cells occurred (Fig 4) In addition distortion of Reissner's membrane was observed which may possibly result in interference with the motion mechanics of the cochlear partition

RESULTS OF COCHLEAR IRRADIATION

The immediate effects of irradiating the cochlea through the round window were observed during examination of surface preparations and consisted mainly of disorganisation of the outer hair cells throughout the basal turn and occasional cell losses in the second turn (Table I) A dosage related effect was seen where 50 milliwatts produced disarray of hair cell cilia restricted to the first cochlear turn while 100 milliwatts resulted in cellular loss and stunted hair cell cilia in the first two turns (Fig 5) The supporting cells in the organ of Corti remained unchanged at the maximum irradiation dosage When examining the cochlea four weeks post-irradiation hair cell damage was more extensive and additional cellular changes occurred throughout the first two turns These included destruction of Deiter s cells and disorganisation of Claudius cells (Fig 6) Also the secretory epithelium on the endolymphatic surface of the stria vascularis showed cellular disorganisation in the basal turn and vacuo-

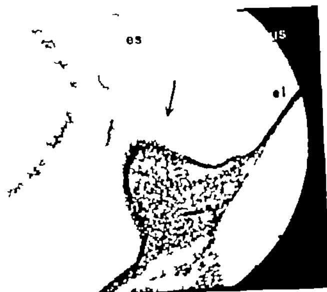


Fig 3 Histological appearance of the crista in the superior semicircular canal ampulla following irradiation into the vestibule in cats. Deterioration of the cupula and cuticular surface of the neuroepithelium was typically most pronounced on the utricular side of the crista (H&E, 500). Cupula, *el* endothelial lining of membranous labyrinth, *scs* semicircular canal side of crista, *us* utricular side of crista.



Fig 4 Vestibular irradiation with 100 milliwatts produced changes than restricted region of the basilar membrane proximal to the round window including loss of outer hair cells (arrows) loss of nuclei from Claudius and outer sulcus cells, and the accumulation of cell debris on the endolymphatic surface of the stria vascularis (H&E, 125).

lation within the second turn Occasionally cellular changes were found to extend into the third turn but these were restricted to vacuolation in and around the hair cells and inner sulcus cells The endolymph contained cellular debris proximal to Claudius cells suggesting a change in permeability

The immediate effect of irradiating the cochlear apex with 50 milliwatts was a disruption of the outer hair cells in the fourth cochlear turn. Increasing the dosage to 100 milliwatts resulted in distortion of the outer hair cells and loss of their cilia from the apeal to the second turn Losses of inner hair cells were mostly restricted to part of the apical turn (Fig. 7) and only occurred following irradiation with the higher dosage The inner hair cells were found to be more resistent to ultrasonic damage as their ciliae loss was restricted to only the apical turn compared to three cochlear turns in the outer hair cells (Table I) Although the outer hair cells were the most sensitive of the sensory cells the degree of damage was too inconsistent to indicate preferential sensitivity of any particular row of hair cells

Table I *Surface preparation results following cochlear irradiation with 100 milliwatts for 20 min applied from the round window and the cochlear apex*

Cochlear turn	Row of hair cells	Condition of hair cells: Round window irradiation	Apical irradiation
I	Outer	Mostly indistinguishable with occasional total hair cell loss but complete loss of cilia	Completely normal with typical W-arrangement of cilia
	Inner	Normal except for disarranged cilia and rare cell loss	Undamaged
II	Outer	Cells intact but cilia disarranged and stunted with occasional losses	No cell losses but irregularities evident in cell outline Disorientation of cilia throughout
	Inner	All cells unaffected with a normal continuous line of stereocilia	Undamaged
III	Outer	All normal with undamaged cilia	Where present the cell walls have an irregular outline Cilia mostly absent and where present show gross deformity in pattern
	Inner	Normal with intact cilia	Irregularities in the row of cilia with occasional break in continuity but cells are intact
IV	Outer	Hair cells and cilia intact	Cells and cilia completely disorganised
	Inner	Hair cells and cilia intact	Almost total destruction of cilia and occasional cell losses

DISCUSSION

Vestibular irradiation resulted in pathological changes in the neuroepithelium and cupulae which would have a significant effect on the functional state of these organs There was no evidence of fistulas or pores in the membranous labyrinth suggesting that any effects on membrane permeability would be of a subtle nature such as by deprivation of energy supply to active transport system The most severe effect on vestibular function was exhibited as a unilateral loss of balance control and was only achieved when ultrasound was directed towards the position of the ampulla of the superior semicircular canal This implies that during the treatment of Meniere s disease vestibular ablation would be most effectively achieved by ensuring irradiation in this direction The mechanism involved in bringing about the functional changes seems to be a destructive effect occurring specifically at the neuroepithelium rather than permeability changes in the endothelium which could be induced by applying ultrasound anywhere within the vestibule

The dosage of ultrasound applied to the vestibule exceeded the damage threshold of neuroepithelial tissue although a relationship between the degree of damage and dosage level could not be determined In general it was found that the dosage of 80 milliwatts applied for 20 min which is typically used in the

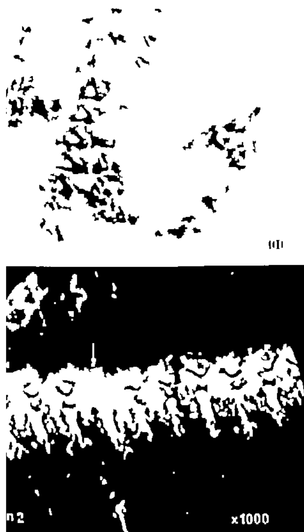


Fig. 5. (A) A surface preparation of guinea pig cochlea showing the normal regular pattern of the outer hair cell cilia (Phase contrast, 1000). (B) Cochlear irradiation with 100 milliwatts resulted in sheeting and distortion of the cilia, and some hair cell losses in the inner most row of the outer hair cells (P.C. 1000). *ihc* inner hair cells, *ohc* outer hair cells, *p* pillar cells.

d window ultrasonic treatment of Me s disease was sufficient to initiate pathological change.

ollowing vestibular irradiation changes in structure were observed in a restricted re of the basilar membrane proximal to the nd window. The resulting impairment of hlear function would involve the response igh frequencies above the range of what is sidered to be practical hearing (Von esy 1960, Stevens et al. 1935). The same asound irradiation regime has produced no deleterious change in the cochlear microphonic response to stimulus frequencies up to 8 kHz (Barnett 1979).

The results of cochlear irradiation demonstrated progressive cellular degeneration whereby the extent of immediate structural changes increased after a post irradiation period of latency during which cellular necrosis became histologically discernible. The immediate histological changes seem to be due to ultrasonically induced cellular agitation rather than a thermal mechanism as they have

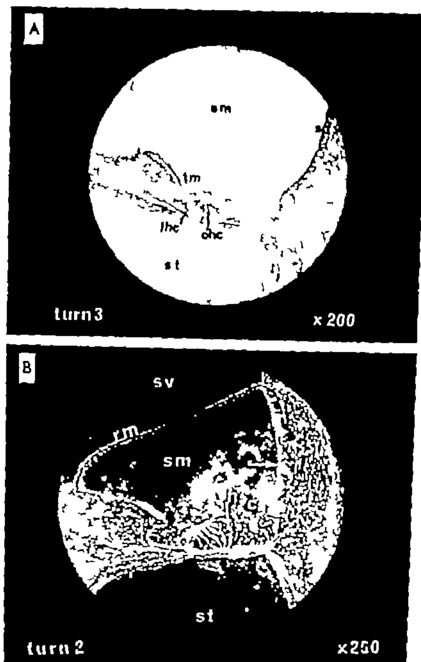


Fig 6 (A) A phase contrast photomicrograph showing the normal cytoarchitecture within the cochlear duct in a control guinea pig (×200) (B) Cochlear irradiation with 100 milliwatts applied through the round window resulted in cellular disorganization within the organ of Corti and vacuolation in the stria vascularis in the first and second cochlear turn (H&E ×250) *c* Claudius cells *h* Hensen's cells, *rm* Reissner's membrane *s* stria vascularis, *sm* scala media *st* scala tympani, *sv* scala vestibuli, *tm* tectorial membrane

not been observed when the intracochlear fluid temperature was raised by an amount equivalent to that occurring during irradiation (Barnett 1979) Although both ultrasound and increased temperature immediately impair hair cell function the thermal mechanism differs by causing cell death without producing immediately visible alterations in cell structure The delayed appearance of such changes contribute towards the observed progressive histological degeneration The end organs of the vestibular and auditory labyrinth are similar in their neuroepithelial composition and relation to labyrinthine fluids and as similar changes in cell structure have been observed in both regions the mechanism involved may be the same During the ultrasonic treatment of Meniere s disease a combination of ultrasonic and thermal mechanisms result in damage at the neuroepithelium of the vestibular end organs.

Following cochlear irradiation the row of inner hair cells was damaged to a much lesser extent than the outer hair cells and a similar effect has also been reported by Crysdale &

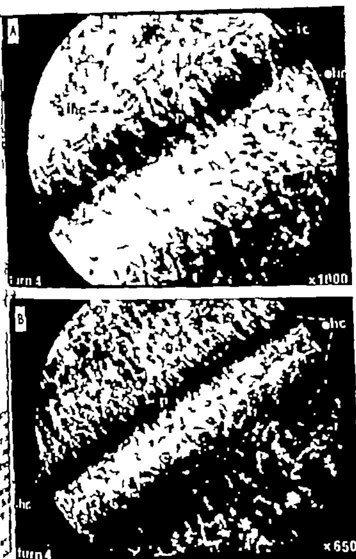


Fig 7 (A) Following irradiation with 50 milliwatts through the bone at the cochlear apex complete loss of outer hair cell cilia occurred together with occasional disruption of the regular row of stereocilia in the inner hair cells (P C., ×1000). (B) Irradiation with 100 milliwatts resulted in cellular disintegration within ohc and some loss of cilia in ihc (P C. ×650). *ic* Inner hair cell cilia.

Stable (1972) when irradiating through the bone overlying the third cochlear turn in guinea pigs It is unlikely that the resistance of the inner hair cells to ultrasound is due to their different physical location as severe damage has been observed in the nearby inner sulcus cells following inner ear irradiation in sheep (Barnett et al 1973)

It was observed that when ultrasound affected the cochlea alterations in the stria vascularis occurred ranging from the presence of cell debris on its endolymphatic surface to cellular disorganisation within its secretory epithelial layer These changes would have a significant effect on the metabolic activity within the organ of Corti particularly as the stria is considered to be an important source of energy within the cochlea. According to Anniko et al (1975) "a degeneration of the stria independent of reason for the degeneration is invariably followed by hair cell damage" and thus may be responsible for the progressive pathology occurring within the cochlea during the post-irradiation recovery period

Therefore apart from the histologically evident destructive nature of ultrasonic energy biochemical changes of this type may represent a more subtle indirect effect

ZUSAMMENFASSUNG

Vestibulare Bestrahlung führte zur Störung der Zellen im Sacculus und im Utriculus aber cochleäre Schädigung war minimal und beschränkte sich auf die Nähe des runden Fensters Funktionsstörung des Gleichgewichtsmechanismus ergab es nur als die Ultraschallwellen direkt auf die Ampulla membranacea superior gerichtet wurden Dies betonte wie wichtig es ist während Behandlung der Ménièreschen Krankheit den Ultraschallstrahl präzis zu richten um maximale Zerstörung der vestibularen Endorgane zu erzielen und um Schädigung auf die Nahe des runden Fensters zu beschränken Cochleäre Bestrahlung zeigte einen merklichen Unterschied an Empfindlichkeit zwischen den zwei Arten von Haarzellen im Organ des Corti wobei die inneren Haarzellen der Ultraschallbestrahlung gegenüber weniger empfindlich waren Das Ausmaß der Haarzellenschädigung war mit der Menge der Bestrahlung verbunden und fortschreitende Degeneration der Zellen geschah im Laufe der Erholungsperiode nach der Bestrahlung

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S B Barnett B.Sc M.Sc
Ultrasonics I stitute
5 Hickson Road
Syd ey N S W 2000
Australia

Acta Otolaryngol 89: 433–439 1980

FATIGABILITY OF THE STAPEDIUS REFLEX IN INDUSTRIAL NOISE

Roland Nilsson, Erik Borg and Gunnar Lidén

From Health and Medical Care Department, Götaverkens Företagshälsovård AB, Göteborg, Department of Physiology II and Department of Audiology, Karolinska Institutet, Stockholm, and Department of Audiology and Otolaryngology, Sahlgren Hospital, University of Göteborg, Göteborg, Sweden

(Received April 19, 1979)

Abstract. Normal hearing subjects were monaurally exposed to 30 min of tape recorded shipyard noise (97 dB (A)) which is characterized by a variable temporal structure. The stapedius muscle activity was continuously recorded in the opposite ear as a change of the ear's acoustic impedance. The reflex function was, in addition, assessed as stimulus-response curves obtained before and, at various times after exposure. A slight reflex fatigue was observed, together with a parallel shift of the stimulus-response curve (average 4 dB). The recovery was slow and not complete even 10 min after the end of the exposure. The individual variability was large. For 5 of the subjects the exposure was repeated at a later session and the individual degree of fatigue was found to be largely predictable. The present results suggest that the stapedius reflex might play a role in the clinical picture of noise induced hearing loss.

Much interest has been focused on the stability and decay of the stapedius reflex response to constant pure tone stimuli and noise signals (Kato 1913; Wersäll 1958; Dallos 1964; Djupesland et al. 1966; Johansson et al. 1967; Tietze 1968; Coles, 1969; Kaplan et al. 1976; Lutman & Martin, 1978; Wilson et al. 1978). These studies all show very rapid adaptation of the response. It is, however, also known that a reflex response which has decayed will reactivate after even a very short pause (Metz, 1951; Wersäll 1958; Borg & Ödman 1979) or after a change in intensity or frequency (Gaevenes & Söhoel 1966). The sound usually resulting in damage to the inner ear—industrial noise—varies with respect to frequency and intensity. It is therefore difficult to predict from available data how the stapedius muscle acts during a workday in an industrial noise environment and thereby the possible role of the reflex in noise induced hearing loss. The presently prevailing opinion is that the muscle rapidly adapts (e.g. Tonndorf 1976) but there are also some early observations (Lüscher 1930; Kobrak et al. 1941) which indicate that this might not be the case. Lüscher observed the stapedius tendon through a perforation in the ear drum. He described a rapid adaptation upon continuous steady sound stimulation but a remarkably great resistance to fatigue in a more varied sound environment.

The aim of the present work was to study the stapedius reflex characteristics in a realistic industrial noise environment. Normal hearing subjects have been unilaterally exposed under laboratory conditions to a 30 min sequence of noise recorded in a shipyard. The stapedius reflex was continuously recorded in the contralateral ear and stimulus-response curves at 0.5 and 2.0 kHz were obtained before and at various times after the exposure. Of particular interest were the time course of reflex recovery after the exposure and individual variability with respect to fatigue. A preliminary report has been published (Borg et al. 1979*a*).

METHODS

The experiments were performed on 18 subjects aged 18 to 47 years. Their hearing thresholds were within 20 dB of ISO 1964 be

The present study was supported by grants from the Swedish Work Environmental Fund (ASF 78/111).

Therefore apart from the histologically evident destructive nature of ultrasonic energy biochemical changes of this type may represent a more subtle indirect effect

ZUSAMMENFASSUNG

Vestibuläre Bestrahlung führte zur Störung der Zellen im Sacculus und im Utriculus aber cochleäre Schädigung war minimal und beschränkte sich auf die Nähe des runden Fensters Funktionsstörung des Gleichgewichtsmechanismus ergab es nur als die Ultraschallwellen direkt auf die Ampulla membranacea superior gerichtet wurden Dies betonte wie wichtig es ist während Behandlung der Ménièreschen Krankheit den Ultraschallstrahl prazis zu richten um maximale Zerstorung der vestibularen Endorgane zu erzielen und um Schädigung auf die Nähe des runden Fensters zu beschränken Cochleäre Bestrahlung zeigte einen merklichen Unterschied an Empfindlichkeit zwischen den zwei Arten von Haarzellen im Organ des Corti wobei die inneren Haarzellen der Ultraschallbestrahlung gegenüber weniger empfindlich waren Das Ausmaß der Haarzellenschadigung war mit der Menge der Bestrahlung verbunden und fortschreitende Degeneration der Zellen geschah im Laufe der Erholungsperiode nach der Bestrahlung

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S B Barnett B.Sc M.Sc
Ultrasonics Institute
5 Hickson Road
Sydney N.S W 2000
Australia

Procedures After otoscopic examination and hearing threshold determination stimulus-response curves were determined for 0.5 kHz and 2.0 kHz pure tones. During the 30 min noise exposure the contralateral response was continuously recorded. The balance of the impedance bridge was continuously controlled and the subject was instructed to swallow repeatedly After the end of the exposure the stimulus-response curves were again obtained according to the following schedule Immediately after the end of the noise 2.0 and 0.5 kHz; after 5 min 2.0 kHz, after 10 min. 2.0 and 0.5 kHz. Finally tympanometry and pure tone audiometry were repeated.

RESULTS

The first and last minutes of the exposure noise were identical This allowed the detection of even minor changes in reflex characteristics. Fig. 1 shows a typical example of such a sequence of recordings The upper graph shows a recording of the noise signal wherein the high intensity periods appear diffuse The middle graph shows the impedance change in the contralateral ear during the first minute and the lower graph shows the corresponding recording during the last minute after 30 min. It is seen that the impedance change well follows the variations in noise intensity It is especially notable that the impact noise elicits a series of responses which summate to give a background level of muscle activity with superimposed peaks The impedance change is altered to some extent after the 30 min exposure Only the lower level components are depressed stronger sounds still give maximum responses

The stimulus-response curves obtained at various times post-exposure were compared with the corresponding curves obtained before exposure in order to quantify the alteration of the reflex and to establish the rate of recovery Fig. 2 shows typical stimulus-response curves before (continuous line) and less than one minute post-exposure (broken line) The stimulus-response curve after exposure is more or less parallel although shifted to the right and the maximum is unchanged A shift of this type is compatible with a selective suppression of the low level activity as shown in Fig. 1

In order to quantify the changes of the reflex the shift of the stimulus-response curve (B in Fig. 2) was measured and expressed as a function of sensation level (A = dB re reflex threshold Fig. 2) The suppression of the stapedius reflex after 30 min noise exposure is described in this way in Fig. 3 Fig. 3 A shows shifts at 2 0 kHz less than one min post-exposure and Fig 3 B shows shifts 0.5 kHz about 2 min after exposure Each subject is shown by a thin continuous line and the mean value ±s e is shown by the heavy line It is seen that the variability is appreciable Some subjects exhibit an improvement of response others suffer more or less pronounced fatigue The reflex depression was significant ($P < 0\ 05$) only at 2 0 kHz (5 and 10 dB re reflex threshold) The average shift is largely independent of the sound level which is indicative of a parallel shift rather than a change of slope of the stimulus-response curve In conclusion there is a small but significant reflex fatigue following an exposure to the 30 min sequence of industrial noise

To investigate the remarkable spread of data seen in Fig. 3 five subjects were reexposed to the same noise from a few days to one year later and retested.

Fig. 4 shows pairs of curves for each subject representing alterations of reflex properties based on 2.0 kHz pure tone stimulation less than one min post-exposure The individual mean values are indicated to the right in the graph It is seen that one subject has consistently large shifts in one direction while another one consistently showed a potentiation of the reflex (negative values) The remaining subjects show intermediate reactions Although no quantitative estimation of reproducibility can be made the results presented

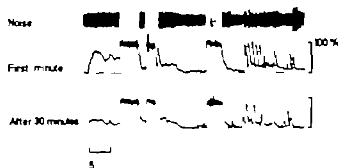


Fig 1 Response of the contralateral stapedius muscle measured as impedance change to shipyard noise Upper graph Exposure noise Middle graph. Imepdance change during the first minute of the exposure Lower graph Impedance change during the 30th min in response to an identical noise sequence Level during this minute 114 dB (A)

tween 0 125 and 8 0 kHz. They had no history of ear disease and their ear drums were normal upon otoscopic examination Reproducibility was assessed by repeated measurements on 5 of the subjects

Recording of stapedius muscle activity The response of the muscle was measured as a change of the ears acoustic impedance (Lidén et al 1972) The 0 8 kHz probe signal (70 dB SPL) was electrically balanced with respect to phase and amplitude The muscle contraction therefore can be measured as the lenght of the complex impedance change vector The stimulus response curve of the reflex was obtained by presenting 1 sec bursts of 0 5 or 2 0 kHz pure tones in the contralateral ear The sound level was raised in 5 dB-steps from 70 dB HL to 120 dB HL and thereafter lowered from 120 to 70 dB HL The middle ear pressure was determined with tympanometry and appropriately adjusted The subjects were instructed to swallow repeatedly during and after the noise exposure to avoid pressure abnormalities

Noise exposure The ear contralateral to the probe ear was exposed to 30 min of industrial noise presented from a tape recorder (Revox A77) via an audiometer (Madsen OB 70) and an earphone (TDH39 MX41AR) In order to minimize interference with the probe tone the exposure noise was fed through a notch filter with a center frequency at 0 8 kHz with a reject band from 0 7–0 96 kHz (General Radio 1952 36 dB per octave)

The tape recording of the noise was done earlier in the welding shop at the shipyard (Nilsson et al 1977) and is typical for the noise exposure of welders and platers. This type of noise contains impulses from sledges and hammers and also high sound levels from pneumatic hammers and striking nut tighteners These impulses are superimposed on a fluctuating background noise level The 30 min portion of the recording used in this test had an equivalent continuous noise level of 97 dB (A)

The first minute of the recording rich in nut tightener noise and impact noise was copied and added to the end of the recording in order to determine fatigue in detail L_{eq} during this minute was 114 dB (A) The equivalent continuous level of the band-reject filtered noise was measured by a 1/1 microphone B&K 4145 6 cc coupler and a noise average meter which integrate the noise level according to ISO standard (*q* fractor=3 dB)

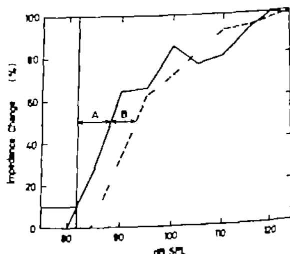


Fig 2 Effect of 30 min industrial noise 97 dB (A) on the stimulus-response curve of the stapedius reflex at 2 0 kHz. Impedance change shown in percentage of maximum obtainable change as a function of sound level The continuous line is obtained before exposure and the broken line less than 1 min post-exposure Threshold is defined as 10% of maximum impedance change and A denotes dB above reflex threshold B is the increase of stimulus level necessary to obtain the same impedance change pre- and post-exposure i e the amount of shift.

Procedures After otoscopic examination d hearing threshold determination, stimulus-sponse curves were determined for 0.5 kHz d 2.0 kHz pure tones During the 30 mm ne exposure the contralateral response was ntinuously recorded. The balance of the pedance bridge was continuously controlled d the subject was instructed to swallow re atedly After the end of the exposure the mulus-response curves were again obtained cording to the following schedule Imme-ately after the end of the noise 2.0 and 0 5 Hz; after 5 min. 2.0 kHz after 10 min. 2 0 d 0.5 kHz. Finally tympanometry and pure ne audiometry were repeated

RESULTS

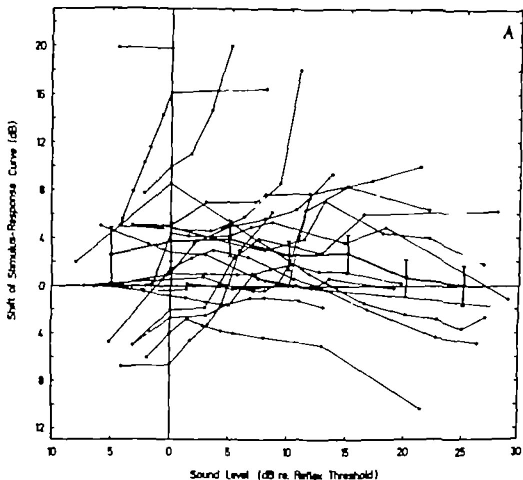
he first and last minutes of the exposure ise were identical. This allowed the detec n of even minor changes in reflex char teristics. Fig. 1 shows a typical example of ch a sequence of recordings The upper aph shows a recording of the noise signal herein the high intensity periods appear dense The middle graph shows the im-pedance change in the contralateral ear during e first minute and the lower graph shows the orresponding recording during the last inute after 30 min. It is seen that the im-pedance change well follows the variations in oise intensity It is especially notable that the mpact noise elicits a series of responses which ummate to give a background level of muscle ctivity with superimposed peaks The im-pedance change is altered to some extent after e 30 mm exposure Only the lower level omponents are depressed, stronger sounds till give maximum responses

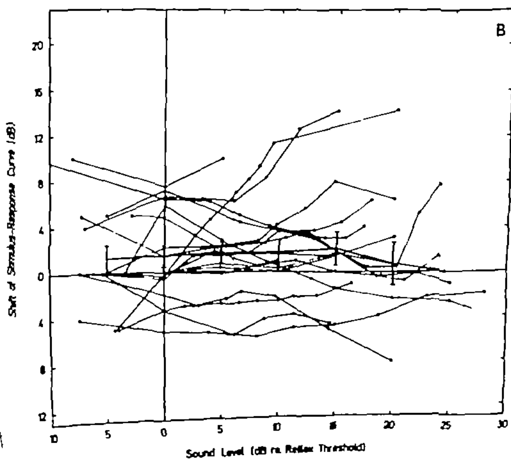
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A
Shift of Stimulus-Response Curve (dB)
Sound Level (dB re. Reflex Threshold)


B
Shift of Stimulus-Response Curve (dB)
Sound Level (dB re. Reflex Threshold)

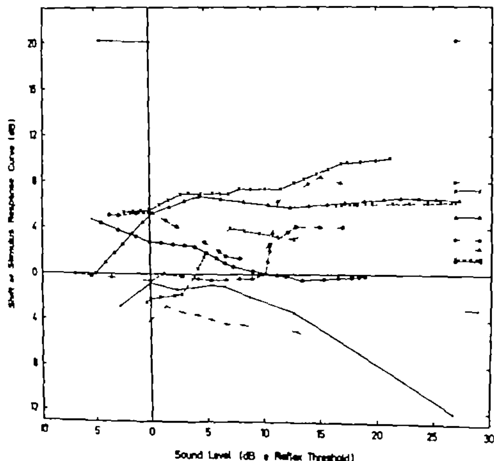


Fig. 4. Reproducibility of stapedius reflex fatigue after 30 min noise (97 dB (A)) obtained in 2 experimental sessions in 5 subjects. Continuous line: first session; broken line: second session. The abscissa shows dB re individual reflex threshold (A of Fig. 2). The ordinate shows the increase in sound level after noise exposure necessary to produce the same impedance change pre- and post-exposure (B in Fig. 2). 0 kHz stimulation, less than 1 min post-exposure. Mean values for each measurement is shown to the right.

in Fig. 3 and 4 seem to reflect a true variability between individuals.

The presentation thus far has been restricted to the reflex suppression immediately post exposure. Although the mean suppression is small, it is possible to see a recovery during the time of observation (10 min). The mean and standard error of the shift of the stimulus-response curves after 30 sec, 5 min and 10 min tested at 2.0 *kHz* is illustrated in Fig. 5. The filled circles represent all 13 subjects and the open circles represent the four subjects with an initial suppression more than 7 dB. There is a recovery which is not complete after 10 min. The time course can be well approximated both with a straight line (broken lines) and with an exponential function (continuous lines).

In conclusion, the stapedius reflex is active

Fig. 3. Shift of stapedius reflex stimulus-response curves after 30 min noise exposure (97 dB (A)) measured as the increase of sound intensity necessary to produce equal impedance changes pre- and post-exposure. The shift ("B" of Fig. 2) is expressed as a function of sensation level re individual reflex threshold ("A" of Fig. 2). Positive values indicate fatigue and negative values potentiation. *A*: test frequency 2.0 kHz. *B*: test frequency 0.5 kHz. Mean values and standard error are indicated by heavy lines.

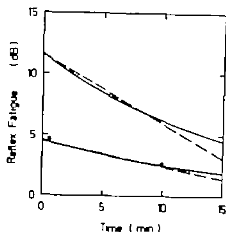


Fig 5 Fatigue of the stapedius reflex obtained as shifts of stimulus-response curves at 2 0 kHz at various times post-exposure Mean values are based on individual shift curves for all 13 subjects (filled symbols) ($y=-0.21x+4$ 5 $y=4.5e^{-\cdot\cdot x}$) and for those 4 subjects with the largest suppression of the reflex (open symbols) ($y=-34.33x+11$ 6, $y=11\ 47e^{\ \cdot\cdot x}$)

throughout a 30 min exposure to industrial noise Generally the response to low level sounds adapts slightly and the individual variability is striking Recovery can be approximated by an exponentially decaying function or straight line with a negative slope

DISCUSSION

The stapedius reflex showed only slight decay after a 30 min exposure to industrial noise recovery however was slow This resistance of the reflex to fatigue found in the present study contradicts the rapid decay during constant pure tone stimulation but confirms earlier observations by Lüscher (1950) and Kobrak et al (1941) A detailed investigation of the decay of the stapedius reflex during stimulation with synthetic complex and time varying sound has recently been published (Luthman & Martin 1978) Very little reflex decay was observed for stimuli of up to 5 min duration (repetitive 50 ms noise bursts with 50 ms intervals) The slow recovery found in the present study is in contrast to the very rapid recovery from decay to short high frequency continuous tones (Borg & Ödman 1979) This does not necessarily indicate however that two separate mechanisms operate. In single unit recordings from auditory nerve fibers, Yound & Sachs (1973) found a decay to continuous pure tone stimulation which recovered more slowly with longer exposures and higher intensities Even though decay to a short sound almost exclusively is a matter of the afferent part of the reflex arc a component of muscular fatigue cannot be excluded from the present result In a parallel field study (Borg et al 1979*b*) with bilateral reflex recordings the muscular function was found to be unaffected after 5–7 h of exposure In addition the slight difference between fatigue at 0.5 kHz and 2.0 kHz favours this interpretation

Although the present findings do not prove that the muscle is active throughout a noisy workday the observed fatigue-resistance supports such an assumption The inter-individual variability observed was striking and some of the subjects showed more fatigue than the majority This suggests that the stapedius reflex might be one source for individual variability in suceptibility to noise induced hearing loss

ZUSAMMENFASSUNG

Achtzehn normalhörende Personen wurden auf einem Ohr 30 min lang mit auf Band aufgenommenem Schiffsbaulärm (Äquivalentniveau $L_{eq}=97$ dB (A)) der in seiner Struktur Zeitlich variabel ist, beschallt. Während der Untersuchungen wurde im Gegenohr die Aktivität des Stapediusmuskels kontinuierlich als relative Impedanzänderung aufgezeichnet Des weiteren wurden auch die Reflexfunktion als Reiz Response Kurve vor und zu verschiedenen Zeitintervallen nach der Beschallung aufgenommen. Als Ergebnis wurde eine leichte Abnahme des Reflexes und eine gleichzeitige Parallelverschiebung der Reiz-Response-Kurve um im Durchschnitt 4 dB beobachtet Der aus der Reiz Response-Kurve ersichtliche Rückgang der Reflexabnahme nach der Beschallung war langsam und noch nach 10 min nach dem Ende der Beschallung nicht vollständig. Allgemein zeigten die Messungen eine erhebliche inter individuelle Variabilität. Bei einer Wiederholung der Lärmbelastung an fünf der Versuchspersonen in einer späteren Sitzung zeigte sich der individuelle Grad der Reflexabnahme weitgehend reproduzierbar Abschließend ist zu bemerken, daß der vorliegende Befund der weitgehenden Ermüdungsresistenz des Stapediusreflexes während der Beschallung vermuten läßt daß er in dem klinischen B ld der durch Lärm erursachten Hörschädigung eine Rolle spielt.

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Professor Gunnar Lidén, M.D.
Dept. of Audiology
Sahlgren Hospital
S-413 45 Goteborg, Sweden

ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CHANGES IN THE GUINEA PIG COCHLEA FOLLOWING MECHANICAL TRAUMA TO THE ORGAN OF CORTI

A R Cody D Robertson G Bredberg[1] and B M Johnston

From the Department of Physiology University of Western Australia Perth Western Australia

(Received November 24 1978)

Abstract Small discrete lesions were produced in the organ of Corti of the guinea pig cochlea using fine probes to produce direct mechanical insult The electrophysiological state of the cochlea was assessed using N_1 electrocochleography and loss of receptor cells determined by scanning electron microscopy Principal findings were 1) Excellent agreement between the location of hair cell losses and the frequency of maximum sensitivity change in the N_1 audiogram 2) The spatial extent of the mechanically induced lesion appears to be more important than the total number of hair cells lost, in determining the magnitude of N_1 sensitivity loss 3) Hair cell losses extending over only 72 μm could be detected as significant changes in N_1 sensitivity These results further emphasize the accuracy and usefulness of the N_1 electrocochleogram for assessing the functional status of the cochlea, 4) Lesions involving only outer hair cell loss also produced marked elevations of N_1 threshold

One traditional approach to the general problem of structure–function inter relationships in the cochlea has been to produce lesions in the organ of Corti and study the subsequent magnitude and extent of the auditory deficit. The results of many of these studies have been paradoxical and anomalous For example hearing losses have been reported accompanied by no apparent damage to receptor cells (Hunter Duvar & Elliott 1972 1973 Dolan et al 1975) Conversely relatively large lesions produce no corresponding hearing loss (Schuknecht 1953 Stebbins et al 1973 Bredberg & Hunter Duvar 1975)

In a recent review Bredberg & Hunter Duvar (1975) pointed out many of the methodological and technical problems which could contribute to such paradoxical findings Limitations exist in both the methods used to produce and evaluate the morphology of lesions and in the techniques chosen to assess the functional status of the cochlea. In particular the use of intense acoustic stimulation to produce receptor cell losses gives generalized effects which render interpretation of changes in cochlear output difficult. Assessment of lesions using light microscopy has the limitation of not revealing fine ultrastructural changes which may be functionally important while serial sectioning can result in spatially restricted hair cell losses not being detected. The measurement of behavioural audiograms in experimental animals is also tedious and has inherent inaccuracies

Our approach has been firstly to produce spatially restricted lesions by direct mechanical insult to the organ of Corti in a reproducible location on the cochlea partition. Secondly the anatomical assessment of these lesions has been performed using high resolution scanning electron microscopy Thirdly we used the thresholds of the gross cochlear nerve action potential in response to tone bursts of varying frequency (N_1 electrocochleogram) to investigate the functional status of the lesioned cochleas There is accumulating evidence that the electrocochleogram is an accurate and sensitive probe of cochlea function (Ruben et al 1976 Johnstone et al 1978 Dallos et al 1978) The present study provides additional support for this notion

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[1] Present address. Department of Otolaryngology Akademiska Sjukhuset S-750 14 Uppsala, Sweden.

MATERIALS AND METHODS

Twenty-one healthy young pigmented guinea pigs (140–400 g) with normal pinna reflex were used in this study. They were anaesthetized intraperitoneally with 50 mg/kg of Pentothal® (sodium pentothal) mounted on rigid hollow ear bars and the entrance to the left bulla gained with a dorsolateral approach under sterile conditions.

A sterile silver wire electrode was placed on the bony shelf near the round window to record the gross neural response (N) to gated tone bursts (rise time 1 msec, 50 msec duration, repetition rate 4 per second). A calibrated closed sound delivery system was used identical with that described by Wilson & Johnstone (1975) the stimuli being delivered to the left tympanic membrane through the ear bar inserted in the external auditory meatus. N responses were amplified by a Grass P15 AC preamplifier (bandwidth 100 Hz–3 kHz) and displayed on a cathode ray oscilloscope (Tektronix 502). An electrocochleogram was obtained by measuring the N threshold (estimated by the visual detection method) for tone frequencies ranging from 2–30 kHz. These thresholds were reproducible to within ±3 dB (Johnstone et al. 1978).

In order to produce a lesion in the organ of Corti the mucoperiostium overlying the basal turn of the scala tympani wall was removed and a small hole (80 μm) hand-drilled in the otic bone with a triangular needle, taking care to avoid the spiral ligament. N thresholds were again measured to check for any damage caused by this initial opening. A glass pipette electrode (20 μm tip diameter) filled with 150 mM KCl was then introduced via the scala tympani opening and passed through the basilar membrane and in some cases Reissner's membrane. The position of the electrode tip was followed by monitoring the d.c. potential difference between the electrode and a neck reference (Keithley 610C Electrometer). The first large negative potential (−60 to −80 mV) indicated entry into the organ of Corti. Appearance of the large +80 to +100 mV endocochlear potential signalled entry into scala media (Johnstone & Sellick 1972). The electrode was then withdrawn and a post-lesion electrocochleogram taken to measure the effect of the mechanically induced lesion on the N_1 thresholds. Small squares of Gelfilm were placed over the hole in the cochlea wall and the opening into the bulla. The skin overlying the bulla was sutured and the animal allowed to recover for periods ranging from 4 to 43 days. No antibiotics were administered.

For the final experiments at the end of the recovery period, the animals were anaesthetized intra-peritoneally with Nembutal® (sodium pentobarbitone 60 mg/kg) and paralysed with Alloferin® (Roche) (4 mg/kg). The trachea was cannulated, the animal placed on ear bars and artificially respirated with carbogen (5% CO_2, 95% O_2).

The bulla was opened via the original surgical entrance and the middle ear examined for signs of otitis media or infection. Two of the animals had middle ear infections and were excluded from this study. In all animals the hole in the cochlea wall had been sealed by sclerotic bone growth.

Post recovery N thresholds were determined using techniques identical with those described above for comparison with the initial and post-lesion N thresholds. Further experimentation consisted of single neurone recordings from the spiral ganglion according to the method of Robertson & Manley (1974). These results are to be reported in another communication.

Both left and right cochleas were then prepared for histological examination. The animal was *decapitated* at the end of the acute experiment and both temporal bones removed rapidly from the skull. The stapes were excised, the round window membranes ruptured and the perilymphatic spaces gently perfused with 3% glutaraldehyde in 0.1 M phosphate buffer within 2 minutes of sacrifice. After 1½ hours in glutaraldehyde the cochleas were rinsed in saline and post-fixed in 1% osmium tetroxide

in 0 1 M phosphate buffer for a further 1½ hours

The specimens were then dehydrated to 70% alcohol and dissected to expose the basal coil of the cochlea

The cochlear wall of scala vestibuli was thinned down using a Kerr Electrotorque drill with a 0 9 mm diamond burr to the level of the basilar membrane and detached The stria vas cularis was then carefully picked away to ex pose the organ of Corti Both Reissner s membrane and tectorial membrane were removed

Each cochlea was photographed in the dis sected state with a Wild M7A stereomicroscope in order to locate the position of any visible lesion (evidenced usually by loss of fibres) in the basal coil Using an eyepiece micrometer (Olympus OSM) measurements were taken to locate the position of the spiral ganglion recording sites in relation to the le sion

After all dissection and measurements had been completed the ligand-linking osmium tetroxide – thiocarbohydrazide method (Malick & Wilson 1975) was used to metal impregnate the specimens which were critical point dried with CO_2 in a Polaron E 3000 critical point dry ing unit (Bredberg 1977) mounted on stubs and examined in a Philips PSEM500 scanning electron microscope (SEM) at magnifications ranging from ×10 to ×7 000

On completion of SEM examination of the organ of Corti the cochlea was further dis sected to expose the hook region This enables absolute distance measurements from the end of the basilar membrane to be made The lesioned area was identified by landmarks noted during scanning microscopy

RESULTS

Pre- and post lesion electrocochleograms

The general shape of the normal N electrocochleogram is shown in Fig. 1 The electrocochleogram consists of a low frequency por

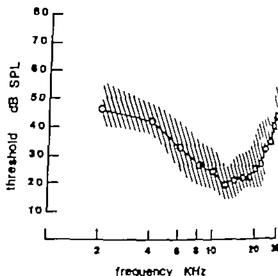


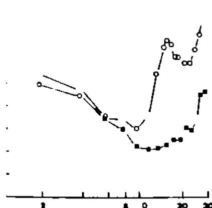
Fig 1 Mean and range for the normal electrocochleogram of the guinea pig (a random sample of 10 from this study) as determined by N threshold sensitivity using the visual detection method

tion with a mean slope of about 20 dB/octave with sensitivity increasing to a plateau region between 12 and 22 kHz. Above 22 kHz, sensitivity falls off at around 40 dB/octave The most sensitive N_1 threshold is at 12 kHz (mean 20 6 S D 1 4 dB SPL) These general features agree well with previous descriptions from our laboratory (Johnstone et al 1978)

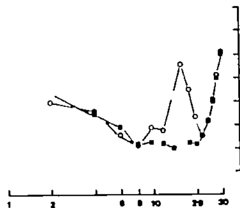
Opening of the scala tympani in preparation for basilar membrane lesioning caused hearing losses in nine of 19 ears The loss in sensitivity was restricted to a narrow range of frequencies and is termed a notch The maximum loss occurred in the range 16–18 kHz (mean 17.2 S D 1 0) The mean magnitude of the loss was 34 7 dB (range 15–79 dB S D 11 1 dB) Two animals (GP59 65 (Fig 2)) showed in addition to the notch a high frequency loss of 26–37 dB at frequencies above 22 kHz In 4 animals no puncture of the basilar membrane was made, whereas in 5 a puncture was made despite the loss on opening the scala tympani

Two sham operations were performed in which successful loss free openings were made in the wall of scala tympani using techniques identical with the above The scala tympani hole was sealed without lesioning the organ of Corti

GP 65



GP 71



frequency KH

T types of hearing loss (■ pre-lesion, O post- induced ben the organ of Corti is mechanically ed The type of loss show by GP71 is termed notch, while GP65 shows in addition a loss in N sensitivity in the higher frequencies.

cturing of the basilar membrane and in cases Reissner s membrane was carried 13 animals Twelve of these (one was completely measured on this occasion) showed an immediate loss of sensitivity e form of a high frequency notch The mal loss of the notch immediately post n (within 5 min) occurred at frequencies ng from 13 to 20 kHz with a mean of kHz (S D 2 0) The magnitude of the loss ed from 9 to 56 dB with a mean of 28.5 dB 15 6)

o significant changes from pre-lesion sholds up to 8 kHz were evident in the -lesion electrocochleogram for all animals he eight ears where no loss occurred on ning the scala tympani the mean loss after cturing was 1 6 dB (S D 13 1 range 5 dB) and occurred at 13–20 kHz (mean kHz. S D 2 5)

four ears showing a loss on opening scala pani the additional effect of puncturing basilar membrane was studied Mean loss ore puncture was 35 0 dB (S D 10 4) at 3 kHz (S D 0 5) and after puncture 4_1 dB D 10 7) at the same frequencies Thus the litional effect of puncturing the basilar membrane on the hearing loss was 7 1 dB (S D 2.7)

Post recovery Electrocochleograms

Recovery period varied from 4 to 43 days. Post-recovery electrocochleograms in animals showing initial hearing loss showed improve ment in post-lesion threshold in all but 2 of the animals Three of the animals had a recovery period of 4–8 days 8 had recovery periods of 17–21 days and 9 had recovery periods of 27–43 days No significant differences occurred in the amount of recovery between these three groups

The 4 animals which showed a loss on opening the scala tympani and in which the basilar membrane was not punctured showed a very small recovery (mean 2.5 dB S.D 5.3 dB) The eight ears which were punctured and did not have a loss on opening scala tympani showed slightly more pronounced recovery (mean 7 9 dB S D 12.0 dB) Three of the animals included in this group showed complete recovery (Fig. 4) The 4 animals which showed loss on opening scala tympani—and in addition the basilar membrane was punctured—

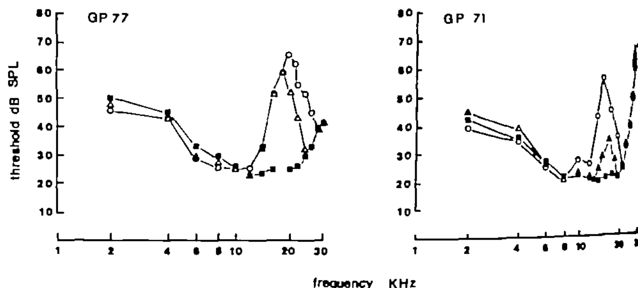


Fig 3 Normal pre-lesion electrocochleogram (■) post lesion (O) and post-recovery (Δ) for GP77 and 71 GP77 showed a loss on opening scala tympani and the organ of Corti was not pierced GP71 showed no loss on opening the scala tympani and the organ of Corti was pierced.

showed the greatest recovery (mean 15 8 dB S D 6.5 dB)

The low frequency slopes of the notch in post recovery electrocochleograms had a mean of 55 1 dB/octave (S D 6 8 dB) while the high frequency slopes of the notch showed a mean of 61 7 dB/octave (S D 9 4 dB) When compared with the post lesion electrocochleogram there was little change in the low frequency slope (52 8–55 1 dB/octave) while the high frequency slope was on average 50% steeper (43 3–61 7 dB/octave) Thus the general effect of recovery was to decrease the frequency range of the notch in the electrocochleogram and also reduce its depth The centre frequency (CF) of the notch after recovery shifted towards a higher frequency in 6 of the animals (max 2 kHz shift) 2 animals shifted to a lower frequency (max 1 kHz shift) and the remainder showed no shift in the CF of the notch

Of the 16 animals which showed initial hearing loss due either to opening of the scala tympani or puncturing of the basilar membrane or both 13 showed a persistent notch with a mean of 27 9 dB (S D 10.9 range 12–40.5 dB) Four of these animals had additional high frequency losses beginning at 20–24 kHz and amounting to 30–46 dB at the most

The four ears which showed loss on opening the scala tympani and were not punctured showed a mean hearing loss of 36 8 dB (S.D. 3 8) The eight ears that were punctured and had no loss on opening scala tympani showed a post recovery hearing loss averaging 13 7 dB (S D 14 4 range 0–40 dB) The four ears that showed loss on opening scala tympani and the basilar membrane was punctured showed post recovery hearing loss averaging 26.4 dB (S D 10 5 range 12.5–38 dB)

No difference in hearing loss either acutely

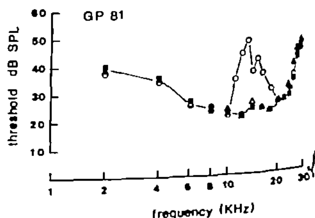


Fig 4 N threshold sensitivity curves (symbols notation as in Fig. 3) showing the complete recovery that occurred after the initial post-lesion hearing loss.

Fig. 5a. Lesioned organ of Corti showing loss of OHCs only one OHC is in the process of degeneration (*arrow*) while the rest of the OHC and the IHCs appear anatomically normal.

-recovery was found between puncture basilar membrane only or in addition re of Reissner's membrane.

emarkable feature of the data was the ex t reproducibility of N thresholds below . Despite the fact that up to 43 days had d between pre-lesion and post recovery rements and despite the difference in of anaesthesia in the two situations pre and post recovery low frequency thresholds were within ±3 dB of each other and often were identical (Figs. 3 4 6 7)

An excellent correlation ($r = 0.84$ significant at 99.9% level) was found between the peak loss in decibels of the post recovery N_1 thresholds and the width in kHz of the notch in the electrocochleogram. Width was measured from the point where high and low frequency slopes intercepted the normal pre-lesion electrocochleogram The two sham-operated gui-

Fig. 5b. High power magnification (×7000) of normal OHC. Stereocilia are tightly packed and regular in their structure



Fig 5c Low-power magnification (×3 500) of OHCs shown in [illegible] stages of degeneration. This animal (GP 75) showed a loss with the opening of scala tympani.

nea pigs showed no significant change in N_1 thresholds between the initial and final measurement of the audiogram with recovery periods of 36 and 38 days respectively

Morphology of the Mechanically Induced Lesion

Of the 16 animals that showed a hearing loss (as estimated by the electrocochleogram) 13 also showed hair cell loss restricted in spatial distribution and located in the basal turn of the cochlea.

The lesions were characterized by a rather sharply demarcated region within which hair cells were either missing or obviously abnormal in their surface structure Changes in the cuticular plates or stereocilia as seen under SEM compared with hair cell structure in normal cochleas from the same weight range of animals were termed abnormal For con-

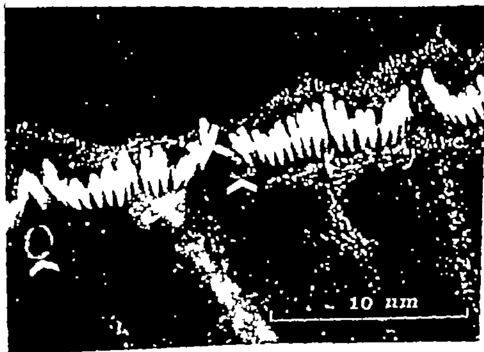


Fig 5d Normal inner hair cells (GP 71) showing regular arrangements of stereocilia and normal distribution of microvilli. Spheroids (arrowed) are probably preparation artefacts.

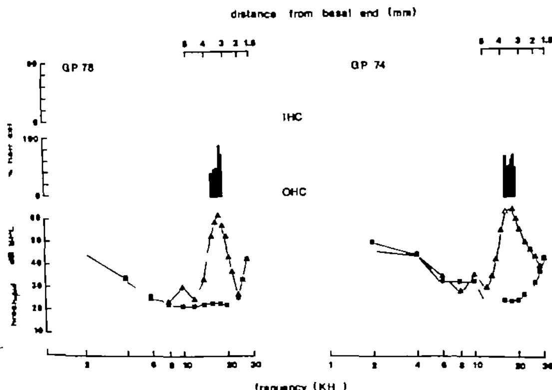


Fig. 4. Upper portion of figure shows hair cell loss (both OHC and IHC) plotted as function of distance from basal end of basilar membrane. Hair cell counts are expressed as percentage loss per 100 µm (or groups of 10 inner hair cells). Lower portion of figure shows the N lectrocochleogram (Δ, post-recovery N thresholds). The relationship between the N thresholds and spatial location of the lesion is explained in the text. GP 74 and 78 both lost only outer hair cells and had bearing loss on opening the scala tympani, the organ of Corti was not pierced.

venience in estimating the size of the lesion abnormal hair cells were scored as absent. Neither of the 2 sham-operated guinea pigs showed hair cell losses or structural abnormalities in the region of the cochlea in question.

The width of these lesions varied from 70 µm to 1.1 mm with an average width of 486 µm. In all but 4 animals both inner and outer hair cells were lost or showed severe abnormalities, but in every case more outer hair cells (OHC) were lost than inner hair cells (IHC). OHC loss generally extended both basally and apically beyond the region of IHC loss.

In the 4 animals in which a loss of N sensitivity occurred on opening the scala tympani, hair cell damage was restricted to the outer hair cells. The loss of OHCs was greatest in the third row and decreased dramatically towards the first (innermost) row. All inner hair cells were present and appeared structurally normal in these animals. An example of SEM results from a lesioned cochlea is shown in Fig. 5. A complete description of the morphology of these lesions is included in a paper in preparation.

Relation between Post recovery Electrocochleograms and Cochlear Morphology

Examples of the type of correlation found between post-recovery electrocochleograms and hair cell loss are shown in Figs. 6 and 7. In these figures the anatomical lesion has been plotted as a function of distance from the basal

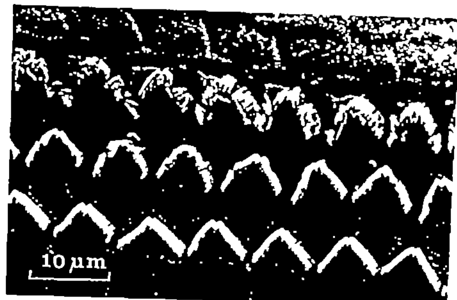


Fig 5c Low-power magnification (×3 500) of OHCs showing various stages of degeneration. This animal (GP 75) showed a loss with the opening of scala tympani

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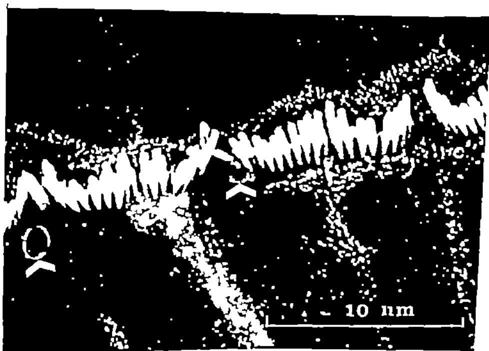


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DISCUSSION

sequence of events following mechanical ıption of the organ of Corti and subent degeneration of the receptor cells is et obscure Introduction of a 20 μm glass e through the cochlea partition in this ly produced loss of hair cells for distances p to 1 1 mm. One possible mechanism ch may result in hair cell degeneration is a ing of the cochlear fluids perilymph and olymph Disruption of the integrity of both ssner's membrane and the cochlea partition ther studies has produced loss of receptor ls similar to that found in this series of anils (Lawrence 1966 Duvall & Rhodes 57 Duvall et al 1969) In our experiments thresholds were not followed for more than min after piercing the organ of Corti and nsequently it was not possible to assess the al extent of the hearing loss until post overy electrocochleograms were meas ed Because the final lesion was in all cases ich more extensive than the size of the obe pure mechanical disruption of the organ Corti seems a remote cause of the final loss the receptor cells. In any case this method s enabled spatially restricted reproducible ss of hair cells to be correlated with the sultant deficiencies in the N electrocochleoram.

The use in this study of SEM rather than erial sectioning and surface preparation (Engtröm et al 1966) allows for a greater degree f accuracy in assessing the morphological ondition of the organ of Corti. In serial sec ioning, as Bredberg & Hunter Duvar (1975) have pointed out, every 10th section is generlly stained and inspected, which means that mall lesions may possibly be missed No such inaccuracy exists in our data Lesions as small as 3 μm (Fig. 5*a*) incorporating only 8 OHCs are clearly visible and the absence of only one hair cell is easily detectable In addition the high resolution of SEM will reveal surface structural abnormalities which may go undetected with light microscopy We have as sumed that obvious abnormalities in cuticular plates and stereocilia imply abnormal func tioning of the hair cell

Several important points emerge from our results Firstly the excellent correlation between location of maximum hair cell loss and frequency of maximum loss of N threshold sensitivity provides an additional validation of the place-frequency map of the guinea pig cochlea, even though our lesions were confined by necessity to a fairly restricted region of the basal cochlear spiral The most complete place-frequency maps have hitherto been derived from basilar membrane mechanical measurements (Wilson & Johnstone 1975) and from spiral ganglion recordings (Robertson & Manley 1974 Johnstone 1977) both methods showing substantial agreement. From data provided by Wilson and Johnstone (1975) the equation

$$f=45e^{-0.771x} \qquad (1)$$

can be derived where f is the cut-off frequency for the basilar membrane tuning curve and x is distance from the basal end of the basilar membrane. Excellent agreement was found in our present data between the frequency of maximum loss in the N electrocochleogram and the location of hair cell loss when these were aligned according to the above equation Thus eq (*1*) appears to reflect accurately the spatial representation of frequency in auditory nerve fibres. The present N data, together with recent spiral ganglion cell recordings (Robertson *et al* in preparation) from our laboratory suggest that the primary nerve fibres at a given location respond best to the frequency corresponding to the high frequency cut-off rather than the peak frequency of the basilar membrane tuning curve at that location (Wilson & Johnstone 1975) This may have im-

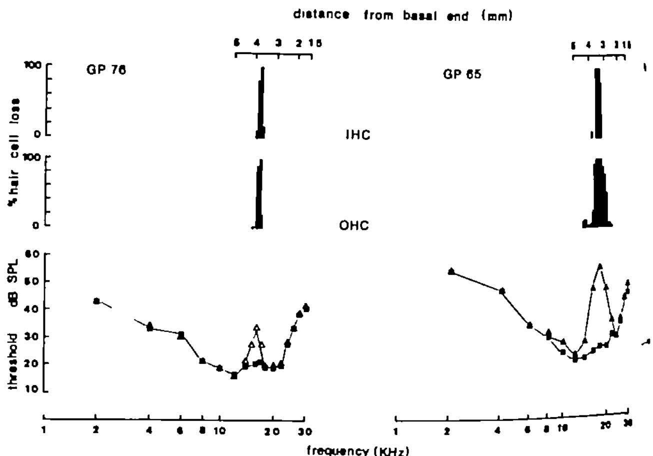


Fig 7 Similar to Fig. 6 but GP76 and 65 both showed a mixed loss of inner and outer hair cells. No loss was incurred on opening scala tympani and organ of Corti was pierced in both cases Δ, Post recovery N thresholds

end of the basilar membrane and then aligned with the lower frequency scale according to the place frequency map of Wilson & Johnstone (1975) and Robertson & Manley (1974)

In all cases the entire anatomical lesion fell within the confines of the notch in the N_1 electrocochleogram All animals showed the greatest percentage hair cell loss occurring at or within 1 5 kHz of the peak of the N_1 threshold loss Fig 7 shows 2 animals which showed mixed inner and outer hair cell lesions Of particular interest are the results shown in Fig 6 This illustrates 2 of the 4 animals which exhibited pure outer hair cell lesions due to scala tympani opening In both these animals only the outermost row of hair cells was missing Once again however the agreement between frequency of maximum N_1 threshold change and the location of hair cell loss is excellent

The peak loss in decibels of N_1 sensitivity shows a much better correlation with the anatomical width of the lesion (r=0 75 significant at 99% level) than with the total number of hair cells lost (r=0 48 not significant) Thus GP78 (Fig 6) in which only 139 hair cells (all outer hair cells) were lost over a distance of 700 μm showed a peak loss at 18 kHz of 40 dB. In contrast GP72 which had 164 OHC and 39 IHCs missing but restricted to a width of 400 μm had a peak loss of only 27 dB at 16 kHz.

In all animals the hearing loss in the electrocochleogram had a greater frequency spread than the anatomical hair cell loss This appears to indicate that although the hair cells either side of a lesion appear to be anatomically normal functionally their thresholds are elevated High power SEM magnifications of hair cells immediately adjacent to the lesion showed no surface abnormalities It is possible that trans-

transmission electron microscopy (TEM) may reveal ultrastructural abnormalities within the actual cell body. No studies of this nature were carried out in this experiment.

DISCUSSION

The sequence of events following mechanical disruption of the organ of Corti and subsequent degeneration of the receptor cells is as yet obscure. Introduction of a 20 μm glass probe through the cochlea partition in this study produced loss of hair cells for distances of up to 1·1 mm. One possible mechanism which may result in hair cell degeneration is a mixing of the cochlear fluids perilymph and endolymph. Disruption of the integrity of both Reissner's membrane and the cochlea partition in other studies has produced loss of receptor cells similar to that found in this series of animals (Lawrence 1966; Duvall & Rhodes 1967; Duvall et al. 1969). In our experiments N_1 thresholds were not followed for more than 30 min after piercing the organ of Corti and consequently it was not possible to assess the final extent of the hearing loss until post-recovery electrocochleograms were measured. Because the final lesion was in all cases much more extensive than the size of the probe, pure mechanical disruption of the organ of Corti seems a remote cause of the final loss of the receptor cells. In any case this method has enabled spatially restricted, reproducible loss of hair cells to be correlated with the resultant deficiencies in the N electrocochleogram.

The use in this study of SEM rather than serial sectioning and surface preparation (Engström et al. 1966) allows for a greater degree of accuracy in assessing the morphological condition of the organ of Corti. In serial sectioning, as Bredberg & Hunter Duvar (1975) have pointed out, every 10th section is generally stained and inspected which means that small lesions may possibly be missed. No such inaccuracy exists in our data. Lesions as small as 30 μm (Fig. 5*a*) incorporating only 8 OHCs are clearly visible and the absence of only one hair cell is easily detectable. In addition the high resolution of SEM will reveal surface structural abnormalities which may go undetected with light microscopy. We have assumed that obvious abnormalities in cuticular plates and stereocilia imply abnormal functioning of the hair cell.

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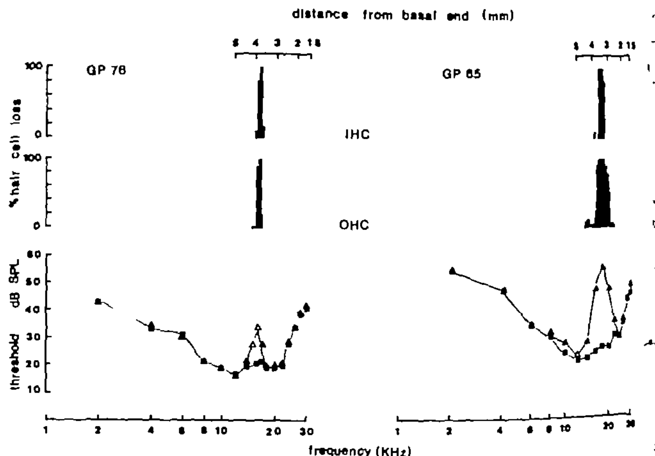


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can be derived where f is the cut-off frequency for the basilar membrane tuning curve and x is distance from the basal end of the basilar membrane Excellent agreement was found in our present data between the frequency of *maximum loss in the N electrocochleogram* and the location of hair cell loss when these were aligned according to the above equation Thus eq (1) appears to reflect accurately the spatial representation of frequency in auditory nerve fibres. The present N data, together with recent spiral ganglion cell recordings (Robertson et al in preparation) from our laboratory suggest that the primary nerve fibres at a given location respond best to the frequency corresponding to the high frequency cut-off rather than the peak frequency of the basilar membrane tuning curve at that location (Wilson & Johnstone 1975) This may have im-

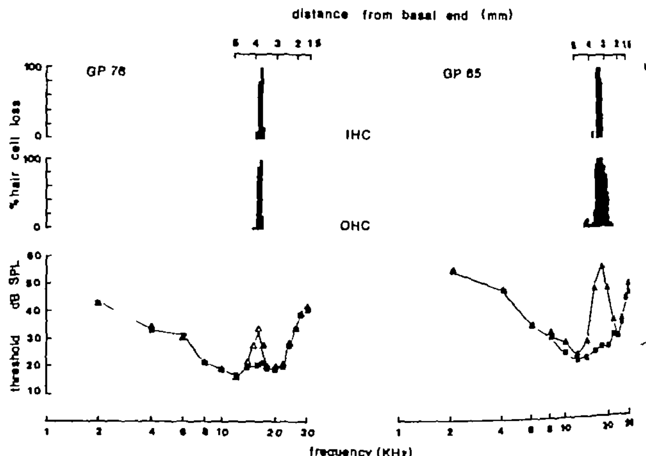


Fig 7 Similar to Fig. 6 but GP76 and 65 both showed a mixed loss of inner and outer hair cells. No loss was incurred on opening scala tympani and organ of Corti was pierced in both cases. Δ Post-recovery N thresholds.

end of the basilar membrane and then aligned with the lower frequency scale according to the place-frequency map of Wilson & Johnstone (1975) and Robertson & Manley (1974)

In all cases the entire anatomical lesion fell within the confines of the notch in the N electrocochleogram All animals showed the greatest percentage hair cell loss occurring at, or within 1.5 kHz of the peak of the N_1 threshold loss Fig 7 shows 2 animals which showed mixed inner and outer hair cell lesions Of particular interest are the results shown in Fig 6 This illustrates 2 of the 4 animals which exhibited pure outer hair cell lesions due to scala tympani opening In both these animals only the outermost row of hair cells was missing Once again however the agreement between frequency of maximum N_1 threshold change and the location of hair cell loss is excellent

The peak loss in decibels of N_1 sensitivity shows a much better correlation with the anatomical width of the lesion ($r=0$ 75 significant at 99% level) than with the total number of hair cells lost ($r=0$ 48 not significant). Thus GP78 (Fig 6) in which only 139 hair cells (all outer hair cells) were lost over a distance of 700 μm showed a peak loss at 18 kHz of 40 dB. In contrast GP72 which had 164 OHC and 39 IHCs missing but restricted to a width of 400 μm had a peak loss of only 27 dB at 16 kHz

In all animals the hearing loss in the electrocochleogram had a greater frequency spread than the anatomical hair cell loss This appears to indicate that although the hair cells either side of a lesion appear to be anatomically normal functionally their thresholds are elevated High-power SEM magnifications of hair cells immediately adjacent to the lesion showed no surface abnormalities It is possible that tran-

authors (Kiang et al 1976 Dallos & Harris 1978 Robertson & Johnstone 1978) that outer hair cells may in some as yet obscure way be necessary for the normal functioning of the afferent nerve fibres which innervate the inner hair cells.

ACKNOWLEDGEMENTS

This work was supported by grants from the Australian Research Grants Council and by the Swedish Medical Research Council, project no 3542. G. Bredberg was supported by a Ramsay Foundation Visiting Professorship. Robertson is Queen Elizabeth II Fellow A Cody is Commonwealth Postgraduate Student.

ZUSAMMENFASSUNG

…tens getrennte Verletzungen wurden am Cortischen Organ der Meerschweinchencochlea erzeugt, indem feine …nden gebracht wurden, um eine direkte mechanische …wundung hervorzubringen. Der elektrophysiologische …stand der Cochlea wurde durch N Elektrocochleographie eingeschätzt und der Verlust an Receptorzellen …rch Abtastung mit Elektronenmikroskop bestimmt. Die Hauptbefunde waren 1) Vortreffliche Übereinstimmung zwischen dem Fokus von Haarzellenverlusten und der Frequenz von Hochstempfindlichkeitswechsel im N …chogrammen. 2) Die räumliche Ausdehnung der mechanisch bewirkten Verletzung scheint wichtiger zu sein als die Gesamtanzahl der verlorenen Haarzellen für die Bestimmung der Hohe des Verlustes an N_1-Empfindlichkeit. … Haarzellenverluste, die sich über nur 77 µm ausdehnten, konnten als bedeutsame Veranderungen der N_1-Empfindlichkeit festgestellt werden. Diese Resultat betonen weiterhin die Genauigkeit und Brauchbarkeit des N_1-Elektrocochleogramms für die Einschätzung der Leistungsfähigkeit der Cochlea. 4) Verletzungen die sich nur auf Verluste der äußeren Haarzellen beliefen, bewirkten auch markierte Erhöhungen der N_1 Schwelle.

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portant implications for the mechanism of frequency coding in the cochlea It is worth noting that our results contrast with numerous studies (outlined by Bredberg & Hunter Duvar 1975) in which the absence of an accurate place frequency map has hampered attempts to correlate hearing loss and cochlear pathology

The N_1 electrocochleogram therefore appears to reflect accurately the functional state of the organ of Corti in that structural damage to a given region is consistently manifested by a deficit in N_1 sensitivity in the expected frequency range Indeed our results highlight the convenience and elegance of the N_1 electrocochleogram in assessing cochlear pathology at least in the range 2–30 kHz as used in this study

Reproducibility of the N_1 threshold measurement and the ability to rapidly test many frequencies contrasts with behavioural audiograms where the time-consuming nature of testing inherent variability in response and limited frequency steps greatly limit accuracy Other recent studies (Johnstone et al 1978 Dallos et al 1978) also attest to the usefulness of the electrocochleogram and its potential importance as a diagnostic tool in clinical assessment of hearing loss in human patients

Other features of the correlation between hair cell loss and N_1 deficits are also interesting The data suggest that it is the spatial distribution of hair cell loss rather than the number of hair cells lost *per se* which determines the resulting magnitude of the N deficit This presumably reflects the spatial spread of the excitation pattern to hair cells A wide lesion means that sound pressure at a particular frequency must be raised to a greater extent to excite distant functional hair cells than in the case of a narrow lesion where functional hair cells are closer spatially to the frequency of maximum excitation In addition two features of the N_1 notch in the lesioned animals suggest that hair cells on the border of the lesion are functionally abnormal though their surface ultrastructure does not differ from that of non lesioned cochleas Firstly the width of the N_1 notch is considerably larger in many cases than the frequency width of the anatomical lesion Secondly if the lesions were ideal, the N_1 notch would have high and low frequency slopes of the same order of magnitude as single unit tuning curves i e greater than 100 dB/octave (Kiang 1965 Evans 1972, Robertson & Manley 1974) No doubt the fact that the edges of our lesions are somewhat diffuse contributes to the findings of notch slopes of the order of 50 dB/octave but once again it is likely that the presence of abnormally functioning hair cells which appear anatomically normal leads to this reduction in the ideal steepness of the notch It is possible that longer survival times are required to allow degeneration of hair cells which have not yet begun to exhibit their occult structural abnormalities

In many of our animals we are unable to exclude the possibility that damage to structures other than hair cells (i e supporting cells and nerve fibres) may contribute to the N_1 deficit. We have made no attempt to quantify alterations in structures other than hair cells.

Undoubtedly the most intriguing results are from animals which showed N_1 sensitivity losses on opening the scala tympani (Fig 6). In all these animals (4 in number) only outer hair cells were missing yet N_1 deficits showed the same characteristics although more pronounced as in deliberately lesioned cochleas with mixed inner and outer hair cell loss The mechanism for these inadvertent lesions is obscure That it could be due to the noise and vibrations when drilling the holes in the scala tympani wall does not seem probable as there was little recovery in the final N_1 electrocochleogram (mean 2.5 dB S D 5 3 4 ears)

The results are clear loss of even just one row of OHCs with apparently normal remaining OHCs and completely intact inner hair cells is sufficient to produce up to a 40 dB peak change in N threshold Since the majority of nerve fibres emanate from IHCs (Spoendlin 1972) it appears as already suggested by van-

Acta Otolaryngol 89: 453–458 1980

PENETRATION OF THE COCHLEAR ENDOST BY THE FIBROUS COMPONENT OF THE OTOSCLEROTIC FOCUS

George Kelemen and Antonio Alonso

From the Ear Research Institute (Eccles Laboratory) and the Otologic Medical Group Inc University of Southern California School of Medicine Los Angeles California USA and the ENT Department of the Special Center Ramon y Cajal Madrid Spain

(Received December 11 1978)

Abstract The role of the "third" "fibrous component of the otosclerotic focus in penetrating and absorbing the cochlear endost is documented. An otosclerotic focus with a fibrous-granulomatous component shows equal power as spongiotic or sclerotic components producing degeneration resulting in rosary-formed absorption of the endost opening access to the soft-tissue containing regions of the cochlea

Penetration of the cochlear endost can be achieved not only by the two components of the otosclerotic focus the spongiotic and sclerotic but also by a third the fibrous-granulomatous mass which is surrounded by the two bony portions (Keleman 1970) This mass is not merely filler material its destructive and penetrating power equals that of the two bony components

The space occupied by this mass can be extensive comprising a considerable part of the focus This space has been identified by various names such as fissula ante and post fenestram resorption foci vascular space and acellular space When the third component surrounds the corner of the stapedial footplate it gives rise to what is called a loose stapes or a floating footplate"

Mayer (1923) considered the wide spaces filled with fibrous marrow to be one of the three forms of otosclerotic organization. Johnson Hawkins and Linthicum 1975 concluded that it was possible for a stapedial ankylosis to exist without an ankylotic stapes i e complete immobilization due to a fibrous mass surrounding the footplate rather than to spongiotic or sclerotic bone overgrowing the angle of the footplate

While discussion of Siebenmann s (1879) theory of the poisonous production of the otosclerotic focus continues (Chevance et al 1970) it is generally agreed that the focus that breaches the cochlear endost and enters the cochelar lumen reaching the soft tissues causes functional damage Replacement of the endost by the focus may result in atrophy of the spiral ligament and of the basilar membrane (Friedmann 1974)

This paper demonstrates the role of the third component at a region of strategic significance the boundary of the cochlear layers of endochrondrium and endost

MATERIALS

Upon examination of 248 temporal bones from 137 patients with otosclerosis in one or both ears 99 were found with the focus reaching the cochlear endost All temporal bones were sectioned in the horizontal plane and stained with hematoxylin and eosin This paper discusses and illustrates 11 examples from the series.

DISCUSSION

In many cases the otosclerotic invasion stopped short of the endost Hough (1964) referred to an invasion that stopped short of the stapedial ringband as hesitation" In this type of invasion it can be assumed that the cochlear endost might be exhibiting a form of self-defense

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A R Cody
Department of Physiology
University of Western Australia
Nedlands
Western Australia



Fig. 4. Wedge-shaped replacement of the periost by sclerotic portion advancing by fingers. ×82.



Fig. 5. Replacement of endost, in rosary formation, by spongiotic focus. ×82.



Fig. 6. Replacement of endost by focus rich in fibrous component. ×52.



Fig 1 Endost destroyed by progressing focus ×172.



Fig 2 Fissure (crack) produced by pressure of the advancing focus rich in fibrous component ×90.



Fig 3 Fissure (crack) caused by pressure of advancing focus extending to the non-otosclerotic osseous mass in the scala. ×82.



Fig. 10. Wedge-shaped replacement, in rosary formation, of endost by focus rich in fibrous component. ×52.



Fig. 11. Wedge-shaped replacement, in rosary formation, of endost by focus rich in fibrous component. ×105.

As demonstrated by many cochlear fractures with intact endost (Kelemen 1944) the endost certainly has resisting power. According to Brunner (1957) otosclerotic bone approaching the endosteum becomes atrophic. Whether avascularity of the endost means defense or vulnerability is questionable. Antoli-Candela et al. (1977) followed the involvement through the cochlear wall, across the spiral ligament, to the basilar membrane. Friedmann (1974) pointed to atrophy of the spiral ligament and rupture of the basilar membrane. According to Linthicum et al. (1975) narrowing of the cochlear lumen and distortion of the basilar membrane are caused by an otospongiotic focus that impinges on the cochlear wall. Benitez & Schuknecht (196_) showed that otosclerosis extending to the endosteum is accompanied by atrophy of the spiral ligament.

The physical force of the focus advancing to the endost is first revealed by a crack or fissure in the endost, as illustrated in Kelemen & Linthicum (1969). Penetration of the endost by the spongiotic or sclerotic portion of the focus is usually wedge shaped, as shown by Manasse (1917).

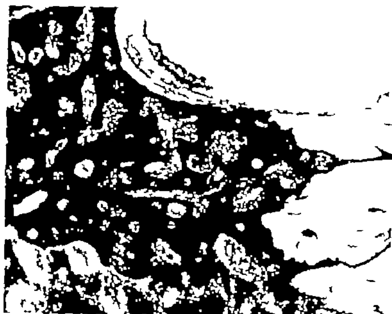


Fig Replacement of endost by focus rich in fibrous component. ×52.

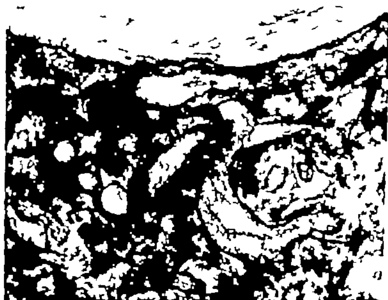


Fig 8 Replacement with uneven surface of the endost by focus rich in fibrous component ×11.

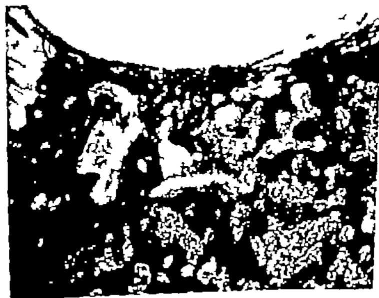


Fig 9 Replacement in rosary-like formation of endost by focus rich in fibrous component ×82.

Acta Otolaryngol 89: 459–464, 1980

THE DIAGNOSIS OF NEGATIVE MIDDLE EAR PRESSURE IN CHILDREN

The Accuracy of Symptoms and Signs Assessed by Tympanometry

T. Lildholdt, J. Courtois, B. Kortholm, J. W. Schou and H. Warrer

From the Department of Otolaryngology, Vejle Hospital, Vejle, Denmark

(Received April 19, 1979)

Abstract. Over a period of one year we studied 357 children at random in order to determine whether or not diagnosis of negative middle ear pressure can be made reliably without using tympanometry. Each child was tested repeatedly by screening audiometry at 20 dB and by tympanometry and was examined once by otoscopy. Information about upper respiratory tract infections, feelings of oppression in the ears, and the parents' opinion of the child's hearing ability were obtained from questionnaires. Otoscopy correlated poorly with the tympanometry, while screening audiometry and the parents' opinion of the child's hearing ability were more reliable measures. It is concluded that tympanometry is an absolutely necessary tool in the clinical diagnosis of negative middle ear pressure.

Negative middle ear pressure, which is the result of an impaired function of the Eustachian tube, occurs very frequently in children (Rendall et al., 1978; Fiellau-Nikolajsen et al., 1977). If it is persistent, secretory otitis media (SOM) may develop, a condition which is characterized by a nonpurulent middle ear effusion. Because the results of today's treatment of SOM are not satisfactory, as stated by Tos & Poulsen (1976), early diagnosis and treatment of this problem, if possible before the effusion has developed, would be desirable.

Impedance audiometry or tympanometry has gained widespread use in otology as a means of documenting the middle ear pressure objectively. The frequency with which this technique is used seems to vary considerably among otologists, and its use is very limited among colleagues in other specialties such as pediatricians and family doctors. Thus, some doctors may claim that the clinical diagnosis of middle ear pathology (i.e. of negative pressure) can be made with sufficient reliability without tympanometry.

In order to evaluate the truth of this assumption it is necessary to examine what is meant by a diagnosis. For this purpose, the elements of the clinical diagnosis may be defined as symptoms and signs. Symptoms are those concomitants of the disease or clues to the disease about which the patient can give positive statements, while signs may be observed or measured by the physician. Thus, the clinical diagnosis may be analysed by evaluating each symptom and sign separately but without analysing the complex interactions of all elements together.

This principle was applied to an epidemiological study, conducted during 1977, of negative middle ear pressure in a randomly selected group of children. The signs of middle ear disease were evaluated from tympanometric, audiometric and otoscopic observations, and some corresponding symptoms of middle ear pathology were obtained from questionnaires answered by the parents.

METHODS

The study involved children from six schools in Vejle, which is an industrial town of about 50000 inhabitants. From 16 classes a total of 357 children were selected, all of whom were

The most conspicuous form of replacement of the endost by the focus was called fragmentation by Kelemen & Linthicum (1969) Before disappearing the endosteal layer is broken into a chain of beads resembling a rosary (Rüedi 1969 Siebenmann 1899 Friedmann 1974) Fragmentation of fibrils producing a rosary like figure was seen by Chevance under the electron microscope

It is shown that otosclerotic foci rich in fibrous elements have the same power as the spongiotic and sclerotic components producing fragmentation resulting in absorption of the endost

ZUSAMMENFASSUNG

Die „dritte" „fibrös-glomerulöse" Komponente des otosklerotischen Focus hat dieselbe Fähigkeit, das Endost zu durchbrechen wie die spongiöse oder sklerose Komponente mit Eröffnung als Zugang zu dem inneren Raum der Schnecke

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G K lemen M.D
Ear R search Institute
256 South Lake Street
Lo Angeles CF 90057
USA

Table III *The clinical validity of the symptoms and signs presented in Tables I and II as indicators of middle ear pathology*

Middle ear pressure indicating threshold of pathology (mmH_2O)	Sensitivity %		Specificity %		Relative liability	
	−160	−240	−160	−240	−160	−240
Upper respiratory tract infection	13.1	14.8	91.1	90.5	1.57	1.77
Otoscopy	54.7	65.3	64.1	63.6	2.16	3.00
Oppression in the ears	07.5	07.5	97.6	97.3	3.01	2.79
Hearing ability	23.2	25.3	93.3	91.7	4.19	3.76
Screening audiogram	1.9	27.2	95.9	95.0	6.62	7.07

naires to be answered by the parents were distributed by the school teachers a few days before each measurement The questionnaire was answered by checking one answer with a maximum of three possible answers For this analysis a distinction was made only between negative and positive answers The number of questionnaires was 1349: thus for 86% of the ears tested data from a corresponding questionnaire was available for comparison

RESULTS

The presence or absence of symptoms of middle ear problems as determined by the questionnaires is presented in Table I The parents were asked if during the last few days the child had shown signs of upper respiratory tract infection and if there had been any complaint of a feeling of oppression in the ears Finally the parents gave their opinion of the child's hearing ability as assessed in the everyday situation in the home The questions did not specify if the right, the left or both ears were involved and therefore the answers were compared with the corresponding mean value of the middle ear pressure of both ears. On the contrary the signs assessed by otoscopy and by the screening audiogram were recorded for each ear separately as shown in Table II In both Table I and Table II the proportions of positive symptoms and signs seen in children with and in those without disease were compared by the χ-square test It appears from the results of the χ-square analysis that a middle ear pressure of less than −240 mmH_2O is not statistically related to the occurrence of upper respiratory tract infection

Sensitivity specificity and relative liability of each symptom and sign were computed in the further analysis of the results The *sensitivity* of the test is the ratio of positive tests in all children with the disease (true positives or TP) to the total number of children who actually have the disease when disease is defined as a middle ear pressure below an indicated threshold (−160 mmH_2O or −240 mmH_2O)

The *specificity* is the ratio of negative tests in all children without disease (*true negatives* or TN) to the total number of children without disease The higher the values of sensitivity and specificity the more reliable is the symptom or sign as an indicator of disease The complement of TP is false-positives (FP) and that of TN is false-negatives (FN). Obviously a diagnostic method with a high value of TP/FP is a good discriminator of disease while a method with a high value of TN/FN is a good discriminator of health In order to compare the effectiveness of different tests as measures of health or disease both of these values must be considered

For practical purposes the formula (TP×TN)/(FP×FN) may be used as a unified measure in the comparison It expresses the *relative liability* of children with disease to show a positive indication of disease compared with the tendency of children free of disease to show positive indications of dis-

Table I *The occurrence of appropriate symptoms in groups of children in whom the mean middle ear pressure of both ears was higher or lower than thresholds of $-160\ mmH_2O$ and $-240\ mmH_2O$*

Mean middle ear pressure in both ears ($-mmH_2O$)	Upper respiratory tract infection			Oppression in the ears			Hearing ability		
	Yes	No	% Yes	Yes	No	% Yes	Impaired	Normal	% Impaired
0–159	97	1 000	8 8	1	840	4	74	1 036	6.7
160–400	28	184	13 0	10	133	7 0	49	16_	_3_
χ square		$p<0\ 05$			$p<0\ 01$			$p<0\ 01$	
0–239	11?	1 109	9 1	?7	9?4	8	101	1 1_3	8 3
?40–400	13	75	14 8	4	49	7.5	22	65	25 3
χ-square		$p>0\ 05$			$p<0\ 05$			$p<0\ 01$	
Total of answers	1_5	1 184	9.5	31	973	3 1	1_3	1 188	9 4

about 7 years old at the time of the study A few children with known sensorineural hearing losses were excluded from the study but those who had been treated for SOM were included

Otologic evaluation of the children was performed in school five times during 1977 Each examination consisted of a screening audiogram to detect hearing losses greater than 20 dB at six frequencies and impedance audiometry to measure the middle ear pressure The Danplex audiometer (TDH 39 headphones) and electroacoustic impedance bridge (220 Hz probe tone) (Model DI 75) were used to determine the presence of a hearing loss and to measure middle ear pressure The middle ear pressure was determined from the air pressure in the external ear canal at the time when the electroacoustic bridge showed minimum impedance (maximum mobility) of the tympanic membrane In the absence of a maximum poin for tympanic membrane mobility the middl ear pressure was recorded as $-400\ mmH_2O$ The children who failed this screening proce dure according to our criteria were referre to the Hearing Clinic which is part of the Oto laryngology Department in Vejle Hospital O the 352 children screened several times 21 children had repeated ear testings in th soundproof room with simultaneous deter minations of middle ear pressure

The present report is concerned with th screening procedure According to the desig of the study 1 760 pairs of ears could hav been tested but for various reasons main absence from school on the day of testing only 1 577 tests were recorded The questio

Table II *The occurrence of appropriate signs in groups of children in whom the middle [illegible] pressure was higher or lower than thresholds of $-160\ mmH_2O$ and $-240\ mmH_2O$*

Middle ear pressure ($-mmH_2O$)	Otoscopy			Screening audiogram		
	Abnormal	Normal	% Abnormal	Abnormal	Normal	% Abnormal
0–159	63	470	35 9	91	15	4 1
160–400	75	6	54 7	84	300	21 9
χ-square		$p<0\ 01$			$p<0\ 01$	
0–_39	289	506	36 4	1__	310	5.0
40–400	49	6	65 3	53	14	7_
χ-square		$p<0\ 01$			$p<0\ 01$	
Total of observations	338	53	38 9	175	45?	6.7

have analysed the prognostic abilities of more specialized methods of otoscopy such as the use of the operating microscope (Axelsson & Lewis 1976) which showed a very high accuracy rate. Roeser et al (1977) compared the assessments of an experienced otologist and a pediatrician each of whom examined the children with a pneumatic otoscope and impedance measurements They found poor agreement between the results of otoscopy and impedance measures as well as poor inter-examiner agreement

The best relative liability was found concerning the parent s assessment of the child's hearing ability and concerning the results of screening audiometry however the sensitivity of these assessments was only about 25 %. The reports in the literature on screening audiometry are difficult to compare as the criteria for abnormality vary In general the results from our study seem to be in agreement with those of most other investigations (Beery et al. 1975 Brooks, 1973)

Only about one-fourth of the measured pathological middle ear pressures were accompanied by positive symptoms and signs. This appears from Fig. 1 as the 3 7 % of the measurements with both features vis-à vis the total of 15 4 % of the measurements that showed pathological middle ear pressures So this simplified analysis shows that the symptoms and signs which we evaluated are not good prognosticators of negative middle ear pressure below -160 mmH_2O besides being a possible precursor of SOM a pressure in that range should be considered a possible hearing handicap (Lildholdt et al 1979) Accordingly the recognition of a negative middle ear pressure is important This study shows the limited value of simple otoscopy in this diagnosis This is especially significant because of the importance attributed to this procedure by non-otologists In fact, more reliable information may be obtained by asking parents about their child's hearing ability or by recording a screening audiogram than may be obtained by otoscopy

This study confirms the conclusion of many other investigators that impedance audiometry is an absolutely necessary tool in the clinical diagnosis of negative middle ear pressure

ACKNOWLEDGEMENTS

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ZUSAMMENFASSUNG

In einer Periode von einem Jahr haben wir 357 zufällig ausgewählte Kinder studiert, um herauszufinden, ob es möglich ist, eine zuverlässige Diagnose eines negativen Mittelohrdrucks zu stellen ohne Tympanometrie zu verwenden Jedes Kind wurde wiederholt durch Screening Audiometrie bei 20 dB sowie bei Tympanometrie untersucht und wurde einmal durch Otoskopie untersucht Auskünfte über Infektionen in dem oberen Respirationstraktum das Gefühl eines Drucks in den Ohren und die Beurteilung der Eltern über das Gehörvermögen des Kindes wurden durch Fragebogen erlangt. Otoskopie bildete eine schlechte Korrelation mit Tympanometrie während Screening Audiometrie und die Beurteilung der Eltern über das Gehörvermögen des Kindes ein zuverlässiges Maß ausmachte Daraus folgert, daß Tympanometrie ein absolut notwendiges Gerät in der klinischen Diagnose eines negativen Mittelohrdrucks ist.

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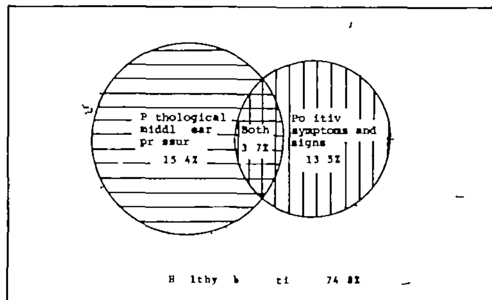


Fig 1 Average occurrence of pathological middle ear pressure (a threshold of −160 mmH_2O is used to designate pathology) and of positive symptoms and signs of pathology in 352 schoolchildren chosen at random *Single measurements* and mean values ha e been combined in this diagram

ease The higher the value of the relative liability the more useful (i e reliable) is the diagnostic method (Abrahamson 1974) In Table III the symptoms and signs are ranked according to their relative liability

Fig 1 gives a general idea of the relationship between pathological middle ear pressures and positive symptoms and signs of this condition In this Venn diagram the intersection of two sets constitutes a small subset where middle ear pathology is associated with positive symptoms and signs suggesting that tympanometry is a prerequisite to identify negative middle ear pressure

DISCUSSION

In this study the analysis of middle ear pathology as a diagnostic problem was made by simplifying the problem Thus any projected use of this system to diagnose middle ear *pathology* in daily clinical practice should be done carefully Furthermore questionnaires were used as the source of patient information while interviews are a very important part of most clinicians assessment and subsequent diagnosis In some respects questionnaires provide information which is superior to that gleaned in an interview as a *larger number of* less biased observations may be tabulated from questionnaires and both the parents and the examiners were unaware of the results of other data gathering tests when *answering* the questionnaires or performing the measurements

As shown by Table III the clinical validity of the symptoms and signs as indicators of disease does not vary much with the cutoff value for pathological middle ear pressure For instance the symptom of oppression in the ears is of limited value in identifying pathology and the presence of an upper respiratory tract infection is of no value in predicting middle ear pathology which also appears from Table I Otoscopy is a sensitive method but its low specificity *reduces* its clinical value It should be noted that otoscopy was performed by experienced otologists but without knowledge of the actual middle ear pressures Otoscopy is in fact the procedure most often used by doctors to diagnose middle ear disease nevertheless we found only one report in the literature which described a comparison of otoscopy with other *methods* of assessing middle ear function (Ferrer 1974) In this report it was not specified whether the otologist knew the actual middle ear pressure but it confirmed the sensitivity of about 50% *found* in our study while reporting a higher specificity rate of about 80% Some authors

…e analysed the prognostic abilities of more …cialized methods of otoscopy such as the … of the operating microscope (Axelsson & …wis, 1976) which showed a very high ac…racy rate. Roeser et al (1977) compared the …essments of an experienced otologist and a …tician each of whom examined the chil… with a pneumatic otoscope and im…dance measurements They found poor …eement between the results of otoscopy …f impedance measures as well as poor in… examiner agreement.

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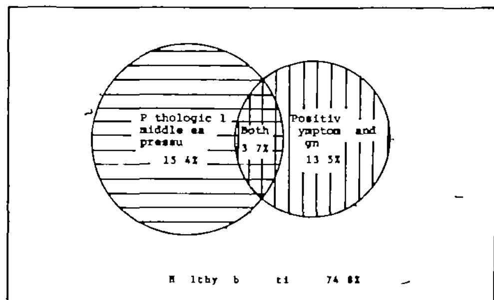


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Fig. 3. Scintigram of patient II presented according to Fig. 1. Increased accumulation in the left mastoid region.

strated an abnormally high accumulation of the isotope corresponding to the right mastoid region and upper lateral part of the right external meatus which areas were left intact at earlier surgery (Fig. 2). The patient was then irradiated with 60 Gy in fractionated doses. A second scintigraphy 2 weeks after the last irradiation dose showed an increase of the pathologic isotope accumulation. The patient died from recurrent carcinoma of the ear and bronchopneumonia in March 1977.

Patient II

A man aged 75 without history, symptoms or signs of chronic otitis. In 1961 a basal cell carcinoma of the skin developed behind the left auricle. The tumour was excised, but from 1966 to 1976 local recurrences appeared and were excised five times. In 1972 the periost of the mastoid process was excised in connection with the skin excision but the bone was left intact. In July 1976 a 4×4 cm tumour was palpated behind the ear, under the skin and against the bone. Histology demonstrated a recurrence and the histopathological diagnosis was changed to squamous cell carcinoma. Radiography did not demonstrate any destruction but tomography demonstrated a destruction of bone under the tumour, probably not explained by the excision of periost in 1972.

Scintigraphy was performed on the same day as the tomography and revealed an abnormally high radionuclide accumulation in the left mastoid region corresponding to the tumour recurrence and the destruction demonstrated by tomography (Fig. 3). The patient was irradiated with 40 Gy in fractionated doses. A second scintigraphy 2 weeks after the last irradiation dose demonstrated an increase in the pathologic isotope accumulation. The patient was operated by radical mastoidectomy, but in February 1979 again a recurrence was verified by histology.

Patient III

A woman aged 72 with symptoms of chronic inflammation of the left middle ear since childhood, with periods of excretion from the ear. A chronic central perforation had been present at least for the last 5 years, but the ear had been dry during this period. Since December 1975 excretion and slight pain had been present in the ear. At examination the internal parts of the external meatus were filled with abnormal soft tissues, possibly engaging the middle ear. No symptoms of cochlear or vestibular engagement were present. Histologic examination of small biopsies revealed a highly differentiated squamous cell carcinoma. Ordinary radiography demonstrated a dense cell system and absence of bone details in the floor of the external meatus.

Tomography of the ear also demonstrated the bone destruction of the floor of the external meatus. The hearing bones could not be identified and the air space of the middle ear

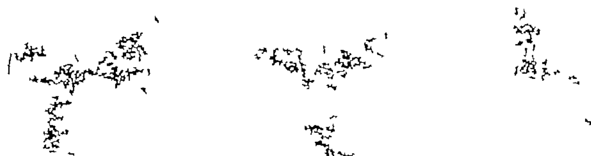


Fig 1 ⁹⁹ᵐTc-DP bone scintigram of the facial skeleton of a control subject RL, right lateral projection LL left lateral projection AP antero-posterior projection

general skeletal survey because of early mammary cancer They did not have any affections of the ear regions nor did they show any symptoms or signs of metastases in the axial skeleton or skull All 10 control cases had a symmetrical distribution of ^{99}Tc^{m}DP in the facial skeleton and in the temporal bones (Fig 1)

Patient I

A man aged 73 complained of excretion from his right ear since December 1975 There was no history of chronic ear affection Clinical examination in March 1976 revealed a tumour 1 cm in diameter in the central part of the auricula and engaging the outer part of the external meatus Ordinary radiography in March 1976 demonstrated a slightly decreased amount of air in the mastoid process on the affected side but no bone destruction However tomography revealed destruction in the upper part of the external meatus

An operation was performed in April 1976 and the auricula the parotid gland and associated soft tissues were excised together with the cartilaginous part of the external ear The mastoid process and the bony part of the external meatus were left intact Histologic examination demonstrated a highly differentiated squamous cell carcinoma growing close to but not into the excision margin against the inner part of the external meatus In July 1976 a facial palsy appeared on the operated side and specimens taken for further histologic examination demonstrated carcinoma tissue in the bony part of the external meatus Tomography of the ear in August 1976 did not demonstrate any change of structure beyond those described in March 1976 Scintigraphy demon-

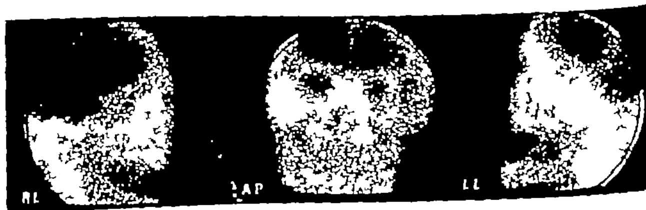


Fig 2 Scintigram of patient I presented according to Fig. 1 Increased accumulation in the right mastoid and outer meatal area.



Fig. 6. Scintigram of patient IV presented according to Figs. 1 and 5. Normal accumulation in both temporal areas. No side difference in AP projection.

ndings at operation could not demonstrate vasive growth of tumour beyond in the walls the external meatus but possibly in the ypotympanon region between this area and e carotid canal. Seven months after surgery recurrence appeared in the operation cavity espite treatment with cytostatic drugs the paent died from the cancer in 1977.

tent IV

A man aged 69 with recurrent excretion from is right ear for 10 years. Examination in 1976 revealed a basal cell cancer of the external meatus engaging the meatus as well as the cranial part of the ear drum. Tomography of the ear did not demonstrate any bone destruction. Scintigraphy was performed in connection to the roentgen examination and demonstrated accumulation of $^{99}Tc^{m}DP$ in both ear regions which were assessed as normal and without side difference (Fig. 6).

No radiotherapy was given. At operation the tumour was found to be growing along the bony walls of the external meatus but no bone



Fig 4 Scintigram of patient III presented according to Fig 1 Increased accumulation in the entire pyramid Central area of low intensity

was replaced by soft tissue No pathologic changes of the inner ear region were demonstrated

Scintigraphy was performed in connection to the roentgen examination and an abnormal accumulation of ^{99m}Tc^{m}DP corresponding to the entire petrous part of the left temporal bone was demonstrated Scintigraphy demonstrated a lower intensity in the central part of the temporal bone than in the surrounding parts (Fig 4) The abnormal accumulation of ^{99m}Tc^{m}DP was better visualized in the oblique lateral projection (L O L) than in the lateral projection (L.L.) (Fig 5) The patient was then irradiated with 40 Gy given in fractionated doses against the area. A second scintigraphy ? weeks after the last irradiation dose demonstrated an increase of the pathologic isotope accumulation

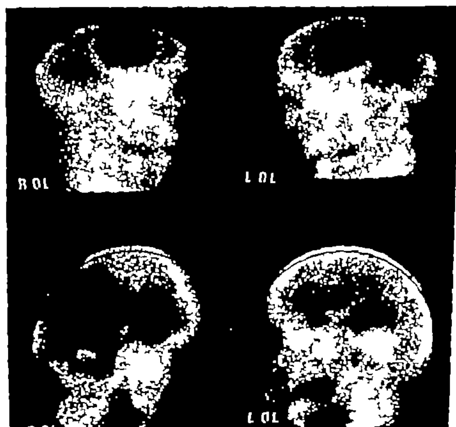


Fig 5 Scintigram of a control subject (*top*) and of patient III (*bottom*) i oblique lateral projections. *ROL* right oblique lateral projection *LOL*, left oblique lateral projection The accumulation in affected left temporal bone more easily visualized. Compare Fig 4

Patient VI

A woman aged 40 suffering from external otitis of the left ear since February 1979 In July a biopsy was taken demonstrating a medium-high differentiated cancer of the ear and the patient was referred for surgery Radiography in August 1979 demonstrated a slightly increased attenuation in the lateral part of the mastoid cell system of the affected ear Polytomography revealed a destruction of the lower part of the external meatus as well as of the temporomandibular joint fossa and the anterior part of the mastoid process

Scintigraphy demonstrated a definitely increased accumulation of ^{99m}Tc^{m}DP in corresponding regions but the area affected according to the scintigraphic results was larger than according to the radiographic results (Fig 8)

The patient was operated one week later and the tumour was found to be growing extensively in the temporal bone and into the parotid region.

DISCUSSION

Five patients (I II III V and VI) with verified squamous cell carcinoma of the ear and bone destruction demonstrated by tomography had abnormally high accumulation of ^{99m}Tc^{m}DP in the temporal area. One patient with a basal cell carcinoma (IV) had no demonstrable bone destruction and a normal bone scintigram The abnormalities were differently shaped in the gamma camera images which indicates a possibility of differentiating between different extensions of neoplastic growth in the temporal bone However inflammatory as well as neoplastic processes influence on bone accumulation of ^{99m}Tc^{m}DP and abnormal accumulation patterns could have several explanations for instance neoplasia, inflammation or both (Greenberg et al., 1968 Galasko & Doyle 1972 Tofe et al 1975 Treves et al 1976 Bergstedt & Haverling, 1978 Bergstedt & Lind, 1978 Bergstedt & Lind, 1979)

The abnormal accumulation in the mastoid region of patient I (Fig. 2) might depend on inflammatory reactions provoked by earlier surgical excision of the auricula and the cartilaginous part of the external meatus or by the small biopsies taken later from this area. However biopsy demonstrated a recurrence of the tumour growth and the mastoid region and the bony part of the external meatus had been left intact by the surgeon It is therefore more likely that the abnormal accumulation was caused by a spread of malignant growth into the cortex of the mastoid process which also explained the destruction demonstrated by tomography

The abnormal accumulation in the mastoid region of patient II was most probably explained by the tumour recurrence verified by histopathological examination which had changed the original diagnosis from basal cell to squamous cell carcinoma. However it cannot be excluded even if not probable that the pathologic uptake of isotope could be explained by the excision of periost of the region in 1977 and therefore this case also illustrates the necessity of careful analysis of scintigraphic findings in correlation to patient history in order to lessen the risk of false-positive diagnosis of neoplastic engagement of bone tissue

The engagement of the whole left pyramid of patient III indicates an influence on bone far beyond the radiologically demonstrable bone destruction of the tumour This patient had a chronic otitis of the left ear and the abnormal accumulation of the isotope might in part be explained by the chronic inflammatory reactions However no acute exacerbations had occurred during the last few years and so the abnormal accumulation was presumably explained by neoplastic influence on the bone Despite the macroscopically radical excision of the tumour area the patient eventually died from local recurrence The scintigraphy findings probably correlated better to tumour extension than did the tomography findings

The basal cell cancer tissue of patient IV



Fig 7 Scintigram of patient V presented according to Fig 1 Pathologic accumulation in the entire right pyramid region.

destruction could be seen Histologic examination verified the diagnosis The patient has been observed without signs of recurrence for 2½ years

Patient V

A woman aged 59 with residual bilateral external otitis for several years Because of a therapy resistant external otitis of the right ear since Dec 1978 she was finally referred to an otologist for treatment The external meatus was compressed by tumour tissue growing within the meatus and infiltrating the parotid region Histologic examination demonstrated a medium-high differentiated squamous cell carcinoma Radiography and polytomography demonstrated destruction of the lateral parts of the bony meatus and the dorsal part of the fossa of the temporomandibular joint. There were neither radiologic nor audiologic signs of affection of the inner ear

Scintigraphy was performed and there was an abnormally high accumulation of ^{99}Tc^{m}DP around the outer meatus in the temporal fossa and in the lateral petrous part of the temporal bone (Fig 7) The patient was treated with irradiation (64 Gy) in fractionated doses A second scintigraphy 2 weeks after the last irradiation dose demonstrated an increase in the pathologic accumulation of the isotope

The patient was operated upon and the cancer was found to be growing around the external meatus and within the middle ear Radical excision had not been achieved according to histologic examination and the patient was further treated with cytotoxic drugs



Fig 8 Scintigram of patient VI presented according to

its extent as well as in evaluating the prog- ss of cancer of the ear They also illus- te the necessity of a careful analysis of patient s history symptoms and clinical lings, in relation to results obtained by er methods This is necessary in order to arate different causes of increased accu- *lation of ^{99}Tc^{m}DP such as chronic or acute* lammation earlier surgery neoplastic wth, etc.

It is not possible at the present level of ex rience to assess completely bone scinti aphy as a method for diagnosing malignan- s of the ear in comparison with other meth ls Further information concerning the Tc^{m}DP distribution in the skull bones of nor al individuals as well as of patients suffering m different malignant inflammatory and her diseases must be investigated in order to epare the necessary basis for adequate inter etation of scintigram results

ZUSAMMENFASSUNG

er potentielle Wert der Knochenscintigrafie für Dia- ostik und Prognosenbeurteilung des Ohrenkarzinoms rd hervorgehoben und illustriert, mit Resultaten von 6 tienten mit hochdifferenzierten Ohrenkarzinom Tcm-Diphosphonat und spezielle Gammakamera-Pro- ktionen wurden angewendet.

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H F Bergstedt M.D
Dept of Diagnostic Radiology
Karolinska sjukhuset
S-104 01 Stockholm
Sweden

extended along the bony walls of the external meatus but no bone destruction could be dem onstrated by tomography or at operation This patient is the only one without demon strable bone destruction and accordingly this patient had a normal accumulation of $^{99}Tc^{m}DP$ He is alive and without signs of re currence at 2½ years after operation

Patient V had an extensive growth of a squa mous cell carcinoma causing bone destruction demonstrable by polytomography in the lateral part of the temporal bone region and a patho-logically high accumulation of $^{99}Tc^{m}DP$ in the corresponding region The tumour growth extended beyond the radioloigcally demon strable destruction and probably corresponded better to the wider extension of the abnor mally high accumulation of $^{99}Tc^{m}DP$

Patient VI also had an extensive growth of squamous cell carcinoma causing radiolog ically demonstrable destruction and at opera tion the tumour growth was found to extend far beyond the limits of demonstrable bone destruction The tumour extension seemed to correspond better to the region of abnormal $^{99}Tc^{m}DP$ accumulation than to the region of radiologically demonstrable destruction

All patients but no IV had squamous cell carcinomas and pathologic accumulation of $^{99}Tc^{m}DP$ in the region affected Furthermore none of the patients but no IV could be sur gically treated with radical excision of the tumour without recurrence despite excision margins far beyond the margins of radiolog-ically visible destruction In contrast patient IV had a basal cell carcinoma without radio-logically demonstrable destruction or abnor mal accumulation of $^{99}Tc^{m}DP$ in the region af fected This patient is the only one with sur gically radical excision still alive without re currence 2½ years after treatment

These findings indicate the prognostic value of temporal bone scintigraphy in combination with radiological examination of patients har bouring carcinomas of the ear The extension of the region of pathologic accumulation of $^{99}Tc^{m}DP$ was in all cases greater than cor responding regions of radiologically demon-strable destruction The findings at surgery and poor prognosis with recurrences in all pa-tients but case IV also indicate that the larger regions of tumour involvement demonstrated by scintigraphic findings should be more rele vant for therapeutic and prognostic assess-ment than the smaller region of destruction demonstrated by radiography However it must be borne in mind that the extension of a hot spot is for physical and technical reasons somewhat larger in the gamma camera image than in reality

The scintigraphic oblique lateral projection, corresponding to the Stenver projection of the ear was found to be of value in examining the pyramid region especially in patient III It is seen from Figs 4 and 5 that this projec tion demonstrated the accumulation and the abnormal side differences more clearly than did the A P and the lateral projections Fur thermore the oblique lateral projection in con-nection with the lateral projection creates dif ferent topographic views of the bone structure and alterations in the orientation of structures in the two projections might give further infor mation on the site and extension of the af fected bone

All scintigrams obtained after radiotherapy of the cancerous ears (patients I II III and V) showed an increase in the abnormal accu-mulation in the particular area in comparison with those scintigrams obtained before radio-therapy This change in bone engagement is difficult to evaluate at present The accumula-tion of $^{99}Tc^{m}DP$ in normal and tumorous bone is reported not to be influenced by radio-therapy per se (Cox 1974 Gates & Gorts, 1976 Lind & Nathansson 1977 Bergstedt & Lind 1979) Thus changes in $^{99}Tc^{m}DP$ ac cumulation might offer information on the progression or regression of malignant growth or inflammatory reactions following irradia-tion

The results presented indicate the potential value of bone scintigraphy in demonstrating the tumorous influence on the bone tissue

I *Patients with prolonged bleeding time*

	Age (years)	Ivy bleeding time (minutes)	Cause of the prolonged bleeding	Treatment
S	13	19	Thrombasthenia	AMCA + fresh whole blood
R	22	18	No definite diagnosis	AMCA
H	22	15	Thrombasthenia	AMCA + fresh whole blood
K.	47	14	Thrombasthenia	AMCA
H	32	14	Enhanced fibrinolytic activity	AMCA
O	49	19	Intake of acetyl-salicylic acid	0
M	52	14	Intake of acetyl-salicylic acid	0
H	5	16	Intake of acetyl-salicylic acid	0
R.	39	16	Intake of acetyl-salicylic acid	AMCA
M	40	15	No definite diagnosis	AMCA
.D	41	17	Unknown	0
E	6[illegible]	18	Intake of acetyl-salicylic acid	AMCA
L	69	16	Unknown	0
L	51	14	Intake of acetyl-salicylic acid	AMCA
M	57	13	Thrombasthenia	AMCA
.V	8	16	No definite diagnosis	AMCA
P	33	18	Intake of acetyl-salicylic acid	0
L	8	14	Unknown	0
C	15	15	No definite diagnosis	AMCA
P	20	14	No definite diagnosis	AMCA
M	9	18	Intake of acetyl-salicylic acid	0
N	55	18	No definite diagnosis	AMCA
S	38	14	Intake of acetyl-salicylic acid	AMCA
O	5	18	No definite diagnosis	AMCA
L	49	18	Intake of acetyl-salicylic acid	0

latelet count was done according to Björk 1 (1959) and Duke bleeding time as de bed by Nilsson (1974) Platelet adhesive s according to Hellem s method (Hellem 0) Aggregation studies of the platelets with m s aggregation method were performed in ayton Dual Channel Aggregation Module as scribed by Cronberg (1970)

Coagulation Coagulation time in glass es and in plastic tubes recalcification time plasma and activated partial thromboplastin e ATP (General Diagnostics) (Nilsson 74) were studied Factor VIII clotting ac ity (VIII C) was determined biologically as scribed by Nilsson et al (1957) factor VIII lated antigen (VIII R Ag) immunochemical m the way described by Holmberg & Nils n (1973) Factor IX one stage prothrombin ne (P&P) factor V fibrinogen factor XIII ibrin stabilizing factor) were determined by ethods reported earlier (Nilsson 1974 lenniksson et al 1979)

Fibrinolysis was tested by determination of fibrinolytic activity of plasma and resuspended *euglobulin precipitate* on fibrin plates euglobulin clot lysis time and fibrin degradation products (FDP) (Nilsson 1974 Nilsson & Olow 1962)

The first Ivy test was performed on the day before operation If the bleeding time was prolonged surgery was usually postponed for 2–3 weeks during which time a re-test and further investigation were performed

RESULTS

In 25 patients (8.3%) we found a prolonged bleeding time (Table I) In 10 cases the condition could be related to intake of acetyl salicylic acid since the bleeding time at re testing after withdrawal of the drug was normal In 3 more cases with no history of acetylsalicylic acid intake the bleeding on repeated assay also was normalized. In the remaining

Acta Otolaryngol 89 474–478 1980

PREOPERATIVE TEST OF BLEEDING TIME IN EAR SURGERY*

S Harris[1] and I M Nilsson[2]

From the ENT Department and [2]Coagulation Laboratory University of Lund Malmö General Hospital Malmö Sweden

(Received June 26, 1979)

Abstract In 300 patients subjected to ear surgery the bleeding time according to Ivy was determined preoperatively In 25 cases prolonged bleeding time was found Further investigations revealed mild thromboasthenia in 4 cases and enhanced fibrinolytic activity in one instance In 10 patients the cause was intake of drugs containing acetylsalicylic acid During operation the patients were treated with AMCA and in two cases fresh blood also was given Even slightly enhanced bleeding can affect the results in microsurgery of the ear We therefore suggest that bleeding time should be determined as a preoperative routine in order to detect the patients in need of specific treatment Patients also should be recommended to avoid drugs containing acetylsalicylic acid for a fortnight before surgery

Good control of the bleeding in the operating field is a pre requisite nowadays for successful microsurgery of the ear The usual ways to achieve this condition are careful local hemostasis various forms of low pressure anesthesia and locally administered adrenalin In spite of these precautions some patients present bleeding problems In order to find this group preoperatively we sought a screening procedure for the detection of disorders of hemostasis e g conditions with defective platelet function decreased factor VIII related antigen levels and high fibrinolytic activity The test of choice seemed to be determination of the bleeding time *ad modum* Ivy The aim of this investigation was three fold viz (1) to detect patients with prolonged bleeding time preoperatively (2) to elucidate the actual disorder and (3) to find the specific treatment during surgery

MATERIAL

The material consisted of two series of altogether 300 patients operated on for various diseases in the middle ear and air cell system. The first 100 patients were subjected to operative procedures which were planned to exceed 2 hours e g mainly cholesteatoma surgery and reconstructive surgery in radical mastoidectomy cavities In the second series, comprising 200 cases all patients subjected to ear surgery were consecutively investigated This means a predominance of stapedectomies and tympanoplastic procedures

Since the results in the two groups did not differ they have been combined There were 140 women and 160 males aged between 3 and 79 (mean age 43) years Patients with known bleeding disorders malignancies liver or renal diseases were excluded from the material

METHODS

The bleeding time was determined *ad modum* Ivy with the modification described by Borchgrevink and Waaler (Borchgrevink & Waaler 1958 Nilsson et al 1963)

A blood pressure cuff on the arm was inflated to 40 mmHg pressure and three transverse cuts were made on the forearm With a special knife Gillette surgical blade E three cuts were made 1 mm deep and 10–14 mm long Mean bleeding time for the cuts was then determined The normal value with this method is 6–12 minutes In patients with prolonged bleeding time further platelet and coagulation and fibrinolytic studies were performed

* This investigation was supported by grants from the Swedish Medical Research Council (no B80-19X-00087 16C)

(	Aggregation in Born aggrego-	Comments
	Pathol	Thrombasthenia
	Pathol.	Thrombasthenia
	Pathol	Thrombasthenia
		Thrombasthenia
		Enhanced fibrinolytic activity
	Normal	No definite diagnosis
	Normal	No definite diagnosis
		No definite diagnosis
		No definite diagnosis
		N definite diagnosis
		No definite diagnosis
	Normal	No definit diagnosis

: effect of acetylsalicylic acid is longstand ;. Even small amounts can give a prolonged eding for up to 2 weeks (Cronberg 1968) condly this substance is a component of my drugs of which the patients do not know : contents In Sweden for instance we can d acetylsalicylic acid in about 60 registered ugs We now cope with this problem by sug- sting drugs containing paracetamol if the pa nts have any need for analgetics during the ntnight before operation.

During the operation the patients received MCA, which is an inhibitor of fibrinolysis. also inhibits the local fibrinolysis thereby reventing the dissolution of haemostatic rombi (Nilsson 1975) As an adverse effect e noted a transient nausea in some patients specially when the drug was given by mouth \s vomiting may be detrimental to the result n reconstructive ear surgery the prophylactic ise of AMCA should be restricted to patients with verified prolonged bleeding time In all he patient with demonstrated thromb- sthenia fresh whole blood or platelet con- centrates was at hand during the operation and had also to be given in cases

With the above-mentioned procedures we achieved satisfactory control of the bleeding in all operations The Ivy test is probably the most sensitive screening method for detecting conditions with prolonged bleeding time In our study the cause could not be revealed in 10 patients and we cannot exclude a false positive test in 3 cases since the re-test gave a normal value

In spite of its simplicity the Ivy test re quires experienced laboratory personnel Recently however General Diagnostics has introduced a device Simplate II™ which gives standardized cuts. A comparison be- tween the results found with Simplate II™ and the method used in our study shows good agreement (Nilsson L. et al in press) This means that Ivy s test could be performed in a satisfactory way in all surgery departments

To ensure a good result in microsurgery of the ear this study thus clearly indicates that the bleeding time should always be deter mined as a preoperative routine in order to detect those patients who need specific treat ment during operation

ZUSAMMENFASSUNG

Vor mikrochirurgischen Eingriffen am Mittelohr wurde bei 300 Patienten die Blutungszeit nach Ivy bestimmt. I 25 Fällen wurde eine Verlängerung der Blutungszeit fest- gestellt. Fernere Untersuchungen ergaben vier Fällen eine milde Thrombasthenie und in einem Fall eine Er hohung der fibrinolytischen Aktivität. Bei zehn Patienten bestand die Ursache in der Einnahme on azetylsalizyl- säurehaltigen Medikamenten Während der Operation wurden die Patienten mit Zyklokaprонsäure (AMCA) behandelt und in zwei Fällen wurde Frischblut erab- reicht

Auch eine geringfügige Steigerung der Blutungen kann das Resultat mikrochirurgischer Eingriff am Ohr nach- teilig beeinflussen Es w rd deshalb vorgeschlagen, als präoperati Routine die Blutungszeit zu bestimmen um die Patienten ausfindig zu machen bei denen eine be sondere Behandlung angebracht ist. Außerdem sollte den Patienten die Einnahme von azetylsalizylsäurehaltiger Präparaten ährend zwei Wochen vor der Operation ab- geraten werden

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Table II *Coagulation and fibrinolytic data*

Case no	APT time (seconds)	Ivy (minutes)	Platelets (10^9/l)	Platelet adh Hellem (%)	AHF (factor VIII) Biolog. (VIII C) (%)	Immunochem (VIII R Ag) (%)	P&P (%)	Fibrinolytic activity on fibrin plates: Citrated plasma (mm²)	Resusp euglob prec (mm²)
1	35	19 17	296	10	110	110	109	0	81
3	33	15	165	8	125	118	–	0	85
4	38	14 15	167	13 1	73	69	11	0	66
15	–	13	290	4	–	–	–	–	
5	35	14 12	181	12 1	100	130	108	0	136
	34	13 18	1 6, 143	10 6	130	139	152	0	78
10	35	15 15	251	6	205	138	170	0	61
16	–	16	norm	norm	–	–	–	–	
19	–	15	230	20	–	–	–	–	
20	39	14 13	239 179	10 4	–	–	–	–	
22	35	18 14	66 198	14 27	130	16_	110	0	81
4	35	18 >20	300 244	30 6 1	143	128	75	26	185
Normal values	<45	6–1	125–340	17–33	60–160	60–175	80–170	4±35	98 54

12 patients the further investigation revealed mild thrombasthenia in 4 cases and a condition with enhanced fibrinolytic acticity in one patient No definite diagnosis could be established in 7 cases (Table II)

Treatment

Sixteen of the patients received AMCA (trans aminomethyl cyclohexane carbonic acid tranexamic acid Cyklokapron®) We used a recommended dose (Andersson et al 1965) of 0 01 g/kg body weight intravenously immediately preoperatively This dose was repeated once or twice on the day of operation In several cases AMCA was also given orally during the postoperative week with a dosage of 1 5 g four times daily Fresh whole blood was available in all the patients with thrombasthenia and had also to be given on two occasions

DISCUSSION

The high incidence of prolonged bleeding time in our study was unexpected

In 4 patients (1 3%) we could demonstrate mild thrombasthenia These patients had a normal number of platelets but prolonged bleeding time and decreased adhesiveness (Cronberg 1968) In 4 more patients who were placed in the group labelled no definite diagnosis because of different or inconsistent findings on repeated investigations mild thrombasthenia could not be excluded While the incidence of the more serious congenital disorders is relatively well known (Nilsson 1976) we have no clear opinion of the frequency of mild thrombasthenia Some observations (Blombäck 1972) however indicate that it might be a common condition

As expected several patients had a prolonged bleeding time due to intake of acetyl salicylic acid (Mielke et al 1969) This inhibits the release reaction of platelets and consequently the second wave of platelet aggregation as measured with Born s aggregometer (O'Brien 1968) In our material 10 patients (4%) had taken aspirin or related drugs This figure should probably have been higher since in the last series we instructed our patients not to take this sort of medicine preoperatively Still it could be proved that intake of acetyl salicylic acid was the cause in several cases. This could be explained by two facts Firstly

Acta Otolaryngol 89: 479–486, 1980

ATTIC RETRACTIONS FOLLOWING SECRETORY OTITIS

M Tos and G Poulsen

From the University E.N.T. Clinic, Gentofte Hospital, Copenhagen, Denmark

(Received May 30, 1979)

Abstract At re-examination of 527 ears with secretory otitis 3 to 8 years after tubulation otomicroscopy and tympanometry revealed different types of retraction of Shrapnell' membrane in 34% of these 4.2% had pronounced retraction with resorption of the osseous annulus and 0.2% had atticus cholesteatoma. There was close correlation between the frequency and severity of retraction on the one hand and the age of the patient at re-examination, the tympanometric conditions, and the pathology of pars tensa on the other The continued progression is further promoted by lack of natural cleansing of the retraction As the frequency of secretory otitis is very high in children the 4.2% of pronounced retractions constitute sufficient quantitative basis for direct progression to atticus cholesteatoma.

The principal purpose of this study was to establish the character and frequency of pathological changes in the Shrapnell's membrane in well-treated secretory otitis Another long-term object has been to investigate whether atticus cholesteatoma is a direct after-effect of secretory otitis or whether it develops directly from retractions in Shrapnell's membrane

Earlier investigations

Several investigators have studied sequelae changes of pars tensa following secretory otitis (Stevens 1962, M Kinnon 1971 Kilby et al 1972, Mawson & Fagan 1972, Kokko 1974 Bonding & Lorentzen, 1973 1974 Tos & Poulsen, 1976) but to our knowledge there exists no systematic investigation of the changes in Shrapnell's membrane

Mawson & Fagan (1972) found attic retractions in 2% of ears after well-treated secretory otitis Kokko (1974) found attic cholesteatoma in 0.7% of ears and McKinnon (1971) in 1.0% when including both attic and sinus cholestea-

In our oldest material comprising patients with secretory otitis treated with tubulation before 1970 and re-examined in 1975 i.e. 5 to 8 years after treatment, we found changes of pars tensa in 55% of ears (Tos & Poulsen 1976) and cholesteatoma in 1% In addition we found different degrees of retractions of pars tensa Unfortunately this material was not systematically investigated with reference to changes in Shrapnell's membrane Consequently in 1977 and 1978 we re-examined a new material of patients operated upon from 1971 to 1975 and after systematic investigations of the changes in Shrapnell's membrane these were divided into predetermined types of retraction.

MATERIAL AND METHOD

The material comprises 527 ears from children under the age of 13 the majority being between 4 and 8 years of age with secretory otitis treated under general anaesthesia with adenotomia myringotomy and exsuction of secretion and most frequently with tubulation.

The children were most often referred from an ENT specialist and our routine method has been to insert an Armstrong tube anteriorly or anterior inferiorly in the drum. In 58 ears tubulation was not performed but only myringotomy Ten ears had much mucous secretion on this side but were not tubulated in deference to the wish of the parents 7 ears had some serous secretion and 31 ears had small or negligible amounts of mucous secretion for which reason we estimated that tubulation was [illegible]ssary The patients were regularly

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Sten Harris
Dept of Otolaryngology
Malmö General Hospital
S-214 01 Malmö
Sweden

Table II *Relationship between age of patient at the re-evaluation and types of retraction of Shrapnell's membrane*

Age years	N of ears	Types of retraction 0 (%)	I (%)	II (%)	III (%)	IV (%)	Cholesteatoma (%)
5 and 6	40	90.0	5.0	5.0	–	–	–
7 and 8	120	79.2	5.8	11.7	1.7	0.8	0.8
9 and 10	130	67.7	13.1	19.2	–		–
11 and 12	137	60.6	18.2	15.3	5.1	0.7	
13 and 14	57	50.9	17.5	28.1	3.5	–	
15 to 20	43	34.9	14.0	30.2	16.2	4.7	–
Total	527	65.7	12.7	17.3	3.4	0.8	0.[illegible]

which could not be seen, in our case (0.2%) it extended to the antrum.

Ret actions and the duration and severity of the disease

There was no relationship between the length of the observation period and the frequency of the different retraction types (Table I). The observation period is the time from the ejection of the *last* tubulus to the time of the re-examination. This is no measure of the duration of the disease since the duration before treatment is unknown. Furthermore children who were retubulated once or twice received treatment over a long period of time and accordingly the observation time was relatively short, in most cases from 3 to 5 years. In the group with an observation period exceeding 5 years, only 16 ears had an observation period of 8 years.

There was a close correlation between the age of the patient at the time of the re-examination and the frequency of retractions (Table II). The percentage of ears with a normal Shrapnell's membrane gradually decreased with age from 90% at the age of 5 or 6 to 35% at the age of 15–20 years (χ^2-test, $p < 0.001$).

The number of retubulations may express the severity or duration of the disease. The frequency of retractions in Shrapnell's membrane was no larger in ears which were tubulated twice ($p > 0.9$) than in those tubulated only once. On the other hand, retractions were significantly more frequent in ears which were tubulated three times than in those tubulated twice ($p < 0.01$) and especially more frequent than in those tubulated only once ($p < 0.001$). The ears which were only treated with myringotomia were, as mentioned, the least affected and Shrapnell's membrane was normal in most cases.

Table III *Relationship between number of tubulations and types of ret action of Shrapnell's membrane*

No. of tubulations	N of ears	Types of retraction 0 (%)	I (%)	II (%)	III (%)	IV (%)	Cholesteatoma (%)
One	380	63.8	13.4	16.1	3.7	0.8	0.3
Two	66	60.6	10.6	25.8	1.5	1.5	
Three	23	30.4	26.1	34.8	8.7		
Paracentesis	58	84.4	5.2	8.6	1.7		
Total	527	65.7	12.7	17.3	3.4	0.8	0.2

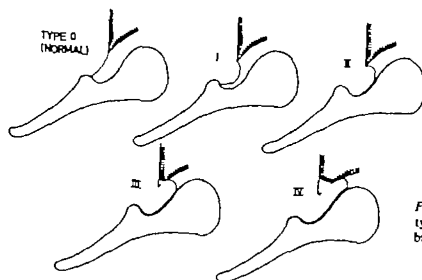


Fig 1 Schematic illustration of different types of retraction of Shrapnell's membrane

checked with audiometry and tympanometry 66 ears (12 5 %) were retubulated once 23 ears (4 4 %) twice The patients were concluded approximately 1 year after ejection of the last tubulus

For re-evaluation in the last 6 months of 1977 and the first 6 months of 1978 tone and speech audiometry tympanometry and otomicroscopy with a Zeiss operation microscope were performed The degrees of Shrapnell s membrane retraction were divided into types from 0 to IV and the character and localization of all pars tensa changes were marked on a drawing of the drum

RESULTS

Definition and frequency

Type 0 indicates a normal Shrapnell s membrane without retraction and with air between the neck of malleus and the Shrapnell's membrane (Fig 1) This occurred in 65 7 % of the ears (Table I) Type I indicates a retraction towards the neck of malleus but with air space visible through Shrapnell's membrane (Fig 1) This type was found in 12.7 % of the ears. Type II indicates that Shrapnell's membrane is retracted to the neck of malleus and that no air space was visible behind the membrane (Fig 1) This type occurred in 17 3 % of the ears Type III indicates that the retraction goes beyond the osseous annulus but the bottom of the retraction may be seen when the head is tilted At the same time there may be a slight bone resorption corresponding to the osseous annulus (Fig 1) This type was found in 3 4 % of the ears Type IV indicates a distinct bone resorption of the osseous annulus and the retraction extends to the head of the malleus However due to the bone resorption the bottom of the retraction may be seen (Fig 1) Atticus cholesteatoma was defined as a deep retraction pocket the bottom of

Table I *Relationship between observation time and types of retraction of Shrapnell's membrane*

		Types of retraction					
Observation time	No of ears	0 (%)	I (%)	II (%)	III (%)	IV (%)	Cholesteatoma
3–5 years	335	65 4	1..8	17 6	3 3	0 6	
5–8 years	19?	66.1	1 .5	16 7	3 6	1 0	
[illegible]	527	65 7	1 7	17 3	3 4	[illegible]	

Table VI *Relationship between pathology of pars tensa and types of retraction of Shrapnell's membrane*

	Types of retraction						
Pars tensa	0 $N=346$ (%)	I $N=67$ (%)	II $N=91$ (%)	III $N=18$ (%)	IV $N=4$ (%)	Cholesteatoma $N=1$ (%)	Total $N=527$ (%)
Normal	57.5	20.9	26.4	5.6	–		45.2
Abnormal	42.5	79.1	73.6	94.5	100.0	100.0	54.8

retraction of Shrapnell's membrane. Diffuse atrophy with retractions of pars tensa is characterized by a thin retracted ear drum with a diminished tympanic cavity but without adhesion to the promontory. Valsalva's test made the drum bulge forward prominently with Siegle's speculum it was hypermobile. Retraction of Shrapnell's membrane occurred in 74% of these ears. In adhesive otitis the drum adhered to the promontory was partly or entirely immobile and the middle ear was partly or entirely atelectatic. There were retractions of Shrapnell's membrane in all ears. Localized atrophy without retraction occurred either in the upper posterior or upper anterior quadrant or downward in tensa. Retraction of Shrapnell's membrane occurred in almost one-third of these ears. Atrophy localized posteriorly with retraction onto the incudostapedial joint and a pexi to the joint was accompanied in 88% of the ears by retraction of Shrapnell's membrane. Diffuse tympanosclerosis or fibrosis of the drum characterized a few ears with a considerably thickened drum, in the remaining ears there was circular or horse-shoe shaped tympanosclerosis in the entire circumference of the pars tensa. Localized tympanosclerosis either posteriorly anteriorly or downwards was in 35% of cases accompanied by retraction of Shrapnell's membrane. A combination of tympanosclerosis in one place and atrophy in another occurred frequently and Shrapnell's membrane was retracted in 66% of these ears.

Table VII shows the clear correlation between the severity of the pathology of pars tensa and the frequency of retractions of Shrapnell's membrane in the following order:

Table VII *Relationship between different pathological conditions of pars tensa and types of retraction of Shrapnell's membrane*

		Types of retraction					
Condition of pars tensa	No. of ears	0 (%)	I (%)	II (%)	III (%)	IV (%)	Cholesteatoma (%)
Diffuse atrophy	3	69	22	9			
Diffuse atrophy and retraction	31	26	19	48	3		3
Adhesive otitis	13		23	38	38		
Localized atrophy	49	69	14	16		0	
Localized atrophy and pexi	37	L.	1	47	18	12	
Diffuse tympanosclerosis	20	45	35	15		5	
Localized tympanosclerosis	79	65	14	19	3	0	
Atrophy and tympanosclerosis	48	44	21	21	13	2	
Total pathology	289	30.9	18.3	43.2	5.9	1.4	0.3
Normal pars tensa	238	83.6	5.9	10.1	0.4		

Table IV *Relationship between amount and character of the effusion and types of retraction of Shrapnell's membrane*

Effusion	Types of retraction 0 $N=346$ (%)	 I $N=67$ (%)	 II $N=91$ (%)	 III $N=18$ (%)	 IV $N=4$ (%)	 Cholesteatoma $N=1$ (%)	 Total $N=527$ (%)
Impacted mucous	71 7	79 1	74 7	72.2	100	100	73 4
Little mucous	25 4	13 4	19.8	16 7	–	–	2_ 4
Serous	9	7.5	5.5	11 1	–	–	4.2

The vast majority of ears (95 8%) had mucous secretion at the first tubulation (Table IV) Among ears with copious mucous secretion there were no significant differences between the frequency of a normal Shrapnell's membrane and the different types of retraction ($p>0$ 1) Among ears with small amounts of secretion there were more ears with a normal Shrapnell s membrane than ears with a type I retraction ($p>0$ 05) though not more than types II or III (Table IV) Thus there is no correlation between the amount or kind of secretion on the one hand and the frequency of Shrapnell s membrane pathology on the other

At the re-evaluation the middle ear pressures were divided into tympanogram types A C_1 C_2 and B (Table V) for practical reasons Considerable middle ear effusion and type B was demonstrated in only 1 7% of the ears while 3% had type B without effusion these had either adhesive otitis or a massive tympanosclerotic ear drum A close correlation was found between the middle ear pressure and the retraction of Shrapnell's membrane Among ears with a type A tympanogram there were significantly ($p<0$ 001) more ears with a normal than with retracted Shrapnell's membrane of all types (Table V)

A significant relationship was found between pathology of pars tensa and retraction of Shrapnell's membrane (Table VI) In the group with a normal pars tensa there were more ears with a normal Shrapnell's membrane and fewer with retractions ($p<0$ 001) More than 78% of ears with retraction of Shrapnell's membrane also had pathology of pars tensa.

The pathological changes of pars tensa were numerous (Table VII) and could be divided into atrophy retraction and tympanosclerosis as well as combinations thereof In diffuse atrophy the ear drum was thin but not retracted Almost one third of these ears had

Table V *Relationship between middle ear pressure at the re-evaluation and types of retraction of Shrapnell's membrane*

Tympanogram type	Middle ear pressure (mmH_2O)	Types of retraction 0 $N=346$ (%)	 I $N=67$ (%)	 II $N=91$ (%)	 III $N=18$ (%)	 IV $N=4$ (%)	 Cholesteatoma $N=1$ (%)	 Total $N=527$ (%)
A	0 to −99	81 7	50 7	45 1	22	75 0		68 9
C	−100 to −199	13 6	26 9	22.0	16 7	25 0		16 9
C_1	−200 to −350	3 2	11 9	25 3	44 4			9.5
B without effusion	Flat	1.2	3 0	4 3	16 7	50 0	100	3 0
[illegible] effusion	Flat	0.3	7.5	3.3	–	–		1 7

Table VI *Relationship between pathology of pars tensa and types of retraction of Shrapnell's membrane*

	Types of retraction						
Pars tensa	0 $N=346$ (%)	I $N=67$ (%)	II $N=91$ (%)	III $N=18$ (%)	IV $N=4$ (%)	Cholesteatoma $N=1$ (%)	Total $N=527$ (%)
Normal	57.5	26.9	26.4	5.6	–		45.2
Abnormal	42.5	79.1	73.6	94.5	100.0	100.0	54.8

retraction of Shrapnell's membrane. Diffuse atrophy with retractions of pars tensa is characterized by a thin retracted ear drum with a diminished tympanic cavity but without adhesion to the promontory. Valsalva's test made the drum bulge forward prominently; with Siegle's speculum it was hypermobile. Retraction of Shrapnell's membrane occurred in 74% of these ears. In adhesive otitis the drum adhered to the promontory, was partly or entirely immobile and the middle ear was partly or entirely atelectatic. There were retractions of Shrapnell's membrane in all ears. Localized atrophy without retraction occurred either in the upper posterior or upper anterior quadrant or downward in tensa. Retraction of Shrapnell's membrane occurred in almost one third of these ears. Atrophy localized posteriorly with retraction onto the incudostapedial joint and a pexi to the joint was accompanied in 88% of the ears by retraction of Shrapnell's membrane. Diffuse tympanosclerosis or fibrosis of the drum characterized a few ears with a considerably thickened drum. In the remaining ears there was circular or horse shoe shaped tympanosclerosis in the entire circumference of the pars tensa. Localized tympanosclerosis either posteriorly, anteriorly or downwards was in 35% of cases accompanied by retraction of Shrapnell's membrane. A combination of tympanosclerosis in one place and atrophy in another occurred frequently and Shrapnell's membrane was retracted in 66% of these ears.

Table VII shows the clear correlation between the severity of the pathology of pars tensa and the frequency of retractions of Shrapnell's membrane in the following order:

Table VII *Relationship between different pathological conditions of pars tensa and types of retraction of Shrapnell's membrane*

		Types of retraction					
Condition of pars tensa	No. of ears	0 (%)	I (%)	II (%)	III (%)	IV (%)	Cholesteatoma (%)
Diffuse atrophy	32	69	22	9			
Diffuse atrophy and retraction	31	26	19	48	3		3
Adhesive otitis	13		23	38	38		
Localised atrophy	49	69	14	16		0	
Localised atrophy and pexi	17	12	12	47	18	12	
Diffuse tympanosclerosis	20	45	35	15		5	
Localised tympanosclerosis	79	65	14	19	3	0	
Atrophy and tympanosclerosis	48	44	1	21	13	2	
Total pathology	289	50.9	18.3	23.2	5.9	1.4	0.3
Normal pars tensa	238	83.6	5.9	10.1	0.4		–

Table IV *Relationship between amount and character of the effusion and types of retraction of Shrapnell's membrane*

Effusion	Types of retraction 0 $N=346$ (%)	I $N=67$ (%)	II $N=91$ (%)	III $N=18$ (%)	IV $N=4$ (%)	Cholesteatoma $N=1$ (%)	Total $N=577$ (%)
Impacted mucous	71 7	79 1	74 7	72.?	100	100	73 4
Little mucous	25 4	13 4	19 8	16 7	–	–	22.4
Serous	9	7.5	5.5	11 1	–	–	4..

The vast majority of ears (95 8%) had mucous secretion at the first tubulation (Table IV) Among ears with copious mucous secretion there were no significant differences between the frequency of a normal Shrapnell's membrane and the different types of retraction ($p>0$ 1) Among ears with small amounts of secretion there were more ears with a normal Shrapnell's membrane than ears with a type I retraction ($p>0$ 05) though not more than types II or III (Table IV) Thus there is no correlation between the amount or kind of secretion on the one hand and the frequency of Shrapnell s membrane pathology on the other

At the re-evaluation the middle ear pressures were divided into tympanogram types A C_1 C_2 and B (Table V) for practical reasons Considerable middle ear effusion and type B was demonstrated in only 1 7% of the ears while 3% had type B without effusion these had either adhesive otitis or a massive tympanosclerotic ear drum A close correlation was found between the middle ear pressure and the retraction of Shrapnell's membrane Among ears with a type A tympanogram there were significantly ($p<0$ 001) more ears with a normal than with retracted Shrapnell's membrane of all types (Table V)

A significant relationship was found between pathology of pars tensa and retraction of Shrapnell's membrane (Table VI) In the group with a normal pars tensa there were more ears with a normal Shrapnell's membrane and fewer with retractions ($p<0$ 001) More than 78% of ears with retraction of Shrapnell's membrane also had pathology of pars tensa

The pathological changes of pars tensa were numerous (Table VII) and could be divided into atrophy retraction and tympanosclerosis as well as combinations thereof In diffuse atrophy the ear drum was thin but not retracted Almost one third of these ears had

Table V *Relationship between middle ear pressure at the re evaluation and types of retraction of Shrapnell's membrane*

Tympanogram type	Middle ear pressure (mmH_2O)	Types of retraction 0 $N=346$ (%)	I $N=67$ (%)	II $N=91$ (%)	III $N=18$ (%)	IV $N=4$ (%)	Cholesteatoma $N=1$ (%)	Total $N=527$ (%)
A	0 to −99	81 7	50 7	45 1	22.2	25 0		68.9
C	−100 to −199	13 6	26 9	22 0	16.7	25 0		16.9
C_1	−200 to −350	3	11 9	25 3	44 4			9.5
B without effusion	Flat	1 2	3 0	4 3	16.7	50 0	100	3 0
B with effusion	Flat	0.3	7 5	3.3	–	–		1 7

Table VI *Relationship between pathology of pars tensa and types of retraction of Shrapnell's membrane*

	Types of retraction						
Pars tensa	0 N=346 (%)	I N=67 (%)	II N=91 (%)	III N=18 (%)	IV N=4 (%)	Chole-steatoma N=1 (%)	Total N=527 (%)
Normal	57.5	26.9	26.4	5 6	–		45.2
Abnormal	42.5	79 1	73 6	94.3	100 0	100 0	54 8

etraction of Shrapnell's membrane Diffuse atrophy with retractions of pars tensa is characterized by a thin retracted ear drum with a diminished tympanic cavity but without adhesion to the promontory Valsalva's test made the drum bulge forward prominently with Siegle's speculum it was hypermobile Retraction of Shrapnell's membrane occurred in 74% of these ears. In adhesive otitis the drum adhered to the promontory was partly or entirely immobile and the middle ear was partly or entirely atelectatic There were retractions of Shrapnell's membrane in all ears Localized atrophy without retraction occurred either in the upper posterior or upper anterior quadrant or downward in tensa. Retraction of Shrapnell's membrane occurred in almost one-third of these ears Atrophy localized posteriorly with retraction onto the incudostapedial joint and a pexi to the joint was accompanied in 88% of the ears by retraction of Shrapnell's membrane Diffuse tympanosclerosis or fibrosis of the drum characterized a few ears with a considerably thickened drum In the remaining ears there was circular or horse-shoe shaped tympanosclerosis in the entire circumference of the pars tensa. Localized tympanosclerosis either posteriorly anteriorly or downwards was in 35% of cases accompanied by retraction of Shrapnell's membrane A combination of tympanosclerosis in one place and atrophy in another occurred frequently and Shrapnell's membrane was retracted in 66% of these ears

Table VII shows the clear correlation between the severity of the pathology of pars tensa and the frequency of retractions of Shrapnell's membrane in the following order

Table VII *Relationship between different pathological conditions of pars tensa and types of retraction of Shrapnell's membrane*

		Types of retraction					
Condition of pars tensa	No of ears	0 (%)	I (%)	II (%)	III (%)	IV (%)	Chole-steatoma (%)
Diffuse atrophy	32	69	22	9			
Diffuse atrophy and retraction	31	26	19	48	3		3
Adhesive otitis	13		23	38	38		
Localized atrophy	49	69	14	16		0	
Localized atrophy and pexi	1	l	12	47	18	12	
Diffuse tympanosclerosis	20	45	35	15		5	
Localized tympanosclerosis	79	65	14	19	3	0	
Atrophy and tympanosclerosis	48	44	l	21	13	2	
Total pathology	289	50 9	18.3	23 2	5.9	1 4	0.3
[illegible]	238	83 6	5.9	10.1	0.4	–	

adhesive otitis atrophy posteriorly and pext diffuse atrophy and retraction of the drum atrophy and tympanosclerosis diffuse and localized tympanosclerosis and finally dif fuse and localized atrophy in which only re tractions of types I and II were found

DISCUSSION AND CONCLUSION

1 Although retractions of types I and II do not present any immediate risk for the patient it is conceivable that over the years some patients may have a progression to types III or IV especially in those with a persisting tubal dysfunction and a negative middle ear pres sure (Table V) Although we were unable to demonstrate any progression in the course of the relative short observation period (Table I) a re-evaluation of the material in some years is absolutely necessary especially since well defined criteria now are available for comparison and since the majority of children at that time will be over 15 years of age As shown in Table II there is a clear tendency to progression of the retraction Shrapnell's membrane when this is related to the age of the patient. Recent screening investigations of healthy children have shown that during the first 2 years of life 50% of all healthy children have had secretory otitis for at least 3 months (Tos & Poulsen 1979) Although a spontane ous normalization of 84% was found a large number of the remaining children will appear for treatment between the age of 4 and 7 years after having had the disease from the second or third year of life In view of this the age of the patient may reflect to some extent the actual beginning of the disease

2 The extremely high frequency of secre tory otitis places in a broader quantitative perspective the 4 4% of ears which in this material had retractions of types III and IV as well as atticus cholesteatomas In most children a slight attack of secretory otitis will clear spontaneously without having been ac knowledged leaving scarcely any after ef fects However because of the very large overall frequency of secretory otitis this group represents a considerable number of children. A hospital material such as this collected over 4 years represents a small percentage of all children with secretory otitis and presumably those with symptoms such as hearing impair ment or acute otitis As shown in Table IV the amount of effusion did not determine the frequency of retraction nor did we find more retractions in children with many episodes of acute otitis before tubulation Therefore it may be assumed that a considerable number of untreated children have the disease in the same degree of severity as in this material and probably the same frequency of retraction of Shrapnell's membrane In a population of 350000 inhabitants 1 100 primary operations for chronic otitis and its sequalae were per formed including a total of 476 choleastomas during a period of 10 years When considering that 152 of these (Tos 1979*b*) had atticus choleastomas then the number of ears with type III and IV retractions must by far exceed the number of atticus choleatomas Thus there is a quantitative basis for the as sumption that atticus choleastomas develop directly from the retractions

3 Earlier we have divided secretory otitis into three stages an initial stage in which the histological changes start a secretory stage in which effusion from the metaplastic mucosa dominates and middle ear secretion accumulates and finally a degenerative stage in which secretion dwindles and earlier formed mucous glands degenerate (Tos & Bak Peder sen 1972 Tos 1976) Several conditions indicate that retractions of the Shrapnell's membrane of types II to IV develop primarily dur ing the degenerative stage i e in the course of the long normalization process of the middle ear mucosa (*a*) in a total of 9 screening investigations (Tos et al 1979) of 1 year-old and 2 year-old children (Tos & Poulsen 1979) including otoscopy of more than 2000 chil dren in which also Shrapnell's membrane was systematically investigated we did not find one single ear with retraction of types II to IV

although 7–14% of the ears at each screening had secretory otitis in the secretory stage and a type B tympanogram furthermore 11–20% had a type C_2 tympanogram. (*b*) At the first tubulation of this material at which time most ears were in the secretory stage with much secretion (Table IV) retraction of types II to IV was not described. However Shrapnell's membrane was not so systematically investigated as at the re-evaluation Yet retraction of types III and IV is so conspicuous that they hardly can be overlooked at otomicroscopy under general anaesthesia. (*c*) In the secretory stage the mucosa is swollen and ickened and the middle ear is more or less lled with secretion. A reversible retraction in pars tensa, will be abolished (*d*) As own in Table II we did not find type III nd IV retractions among 5- and 6-year-old hildren

4 The pathogenesis in the progression of etraction may differ but the primary cause of n incipient retraction is probably a dysfunc ion of the Eustachian tube and the negative iddle ear pressure always present in secre ory otitis When using air-equalization meth- ds, the tubal function is found to be poor luring tubulation and prior to extrusion of the ubulus in 90% of the ears (Renvall & Holmquist, 1974 Poulsen & Tos 1977) The tubal function normalizes gradually so that the middle ear pressure is normal after 1–1½ years in 43–50% (Renvall 1975 Tos & Poulsen 1977) after 1–5 years in 61% and after 5–8 years in 68% of the ears (Tos & Poulsen 1977) Table V shows that also in this material the middle ear pressure is reduced in more than 30% of the ears and that the majority of retractions especially the most severe ones are found among these ears. In type I retractions flaccidity of the Shrapnell's membrane dominates with the possibility of further retraction In types II III and IV the dominant finding is adhesion of Shrapnell's membrane to malleus and here the natural cleansing of the retraction pocket will influence the progression. Accumulation of detritus in the retraction causes inflection which will promote bone resorption of the osseous annulus This we have observed in several ears with type III and IV retractions Even if the middle ear pressure were to normalize a progression of the retrac tion might continue due to the lack of natural cleansing The progression of types II and III will be favoured by a prolonged reduced middle ear pressure or when adhesions are formed between epitympanum and mesotympanum and when the ventilation of epitympanum and antrum is blocked

5 There is a close correlation between the changes in pars tensa and Shrapnell's membrane (Table VII) and especially in ears in which the effect of a prolonged low pressure in pars tensa has dominated as in adhesive otitis in atrophy with retraction and pexi as well as in diffuse atrophy and retraction The largest percentage of ears was found with a pathological Shrapnell's membrane which supports the above mentioned pathogenetic considerations

ZUSAMMENFASSUNG

Bei otomikroskopischer und tympanometrischer Nachuntersuchung der 527 Ohren mit sekretorischen Otitis wurden 3–8 Jahre nach der Tubulation bei 34% der Ohren verschiedene Grade der Retraktionen der Pars Flaccida gefunden. Bei 4,2% der Ohren waren die Retraktionen schwer mit Resorption der knöchernen Annulus, und 0,[illegible]% hatten Cholesteatom im Atticus Es wurde eine deutliche Korrelation zwischen der Häufigkeit und dem Schwerbeitsgrad der Retraktionen auf der einen Seite und zwischen Alter der Patienten bei der Nachuntersuchung, Tympanometrieverhältnissen und Pathologie der Pars Tensa auf der anderen Seite gefunden. Die Progression der Retraktion wird von gestörter Selbstreinigung der Retraktion befördert. Da die Häufigkeit der sekretorischen Otitis bei Kindern sehr hoch ist, bilden das 4,[illegible]% der gefundenen schweren Retraktionen eine genügende quantitative Basis für eine direkte Progression der schweren Retraktionen zum Atticuscholesteatom.

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M Tos M.D
E.N T Department
Amtssygehuset i Gentofte
DK 2900 Hellerup
Denmark

Acta Otolaryngol 89 487–496 1980

CERVICO-OCULAR REFLEX IN THE NORMAL ADULT

David Barlow and William Freedman

From Temple University Department of Rehabilitation Medicine Rehabilitation Engineering C nter Moss Rehabilitation Hospital Philadelphia USA

(Received April 4 1979)

Abstract (1) The cervico-ocular reflex (COR) in humans is measured hile the subjects (=10) stood on ro-tatable platform in dark room with the head fixed by stationary biteplate Eye movements were measured in response to active and passive rotations about vertical axis (2) The COR gain (l horizontal eye movement/amplitude of body rotation) was as great as 22% at low frequency of body rotation (0 025 Hz) With increasing frequency (g., at 0 4 Hz) the gain decreased to about 7 (3) The phase angle of the eye movement ranged generally between −80 and 240 degrees with an average response around 180 degrees (4) During active rotation the COR response was similar to the responses measured ing passive body rotation (5) The principal conclusion is that in normal adult humans the COR does not in stabilization of the image on the retina during pas-e or active body rotations. A theoretical function for COR is presented and discussed

rly this century Bárány (1906) reported that could observe reflexive eye movements in bbits by fixing the rabbit s head and rotating trunk about various axes He concluded at the reflex was tonic since the eyes re ained in their deviated position as long as ck torsion was maintained Bikeles & Ruttin 915) were the first to report similar reflexive e movements in man. They described hav produced compensatory eye movements in 18-year-old male with inexcitable laby nths by rotating the man s trunk while hold-g his head fixed Bárány (1918) disclosed that could produce similar eye movements in wborns and premature infants These re xive eye movements could only be observed ring the first two days after birth after hich time they became obscured by spon-neous voluntary eye movements

The non-vestibular origin of this reflex was recognized quite early but it was not until thirty years later that the receptor sites were convincingly shown. The strongest evidence (Biemond & deJong 1969 Hikosaka & Maeda, 1973 McCouch et al 1951) suggests that the receptors involved with the cervico-ocular reflex (COR) reside in the interverte bral joints and ligaments of the first three cer vical vertebrae

Although cervically induced eye movements were commonly seen in experimental animals and could be observed in infants and in adults under certain pathological conditions they were never seen in the normal adult (DeKleyn & Stenvers 1941) After considerable debate over whether the reflex actually existed in nor mal adults its presence was finally demon-strated when researchers began testing sub-jects in the absence of visual feedback (Philips-zoon 1962 Philipszoon & Bos 1963 Suzuki 1972, Takahashi & Oyster 1974) Visual feed-back was commonly eliminated by placing the subject in a dark room or having him close his eyes

Fixation of the head together with the re moval of visual input allows isolation of the COR from the two other primary oculomotor reflexes—the vestibulo-ocular reflex (VOR) which responds to head acceleration (Melvill Jones & Milsum, 1965) and the opto-kinetic reflex (OKR) which responds to retinal image slip (Takahashi & Oyster 1974) It has been presumed that the COR acts together with the VOR and OKR to stabilize images on the retina during active head movements (Dich-

gans et al 1973 Fredrickson et al 1966 Hikosaka & Maeda, 1973 Hughes 1971 Meiry 1971 Suzuki 1972) An example of this oculomotor interaction was noted when Dichgans et al (1973) showed in the monkey that the cervico-ocular response increased dramatically after bilateral labyrinthectomy However in humans there have been only two investigations indicating measurements which could verify the presumption that the COR actually acts to stabilize the retinal image in association with the VOR Both of these have reported the gain and phase characteristics of eye movements (response) with respect to body movements (stimulus)

Takemori & Suzuki (1971) reported a gain of approximately 8% for rotations at 0 25 Hz Meiry (1971) reported a gain of 30% for frequencies below 0 1 Hz which then gradually decreased to around 8% for frequencies about 0 4 Hz Meiry also observed that the phase of the eye movements would aid in image stabilization Takemori & Suzuki claimed that contrary to common beleif the COR response in the normal adult would not aid in image stabilization—at least at 0 25 Hz the only rotational frequency which they used

To resolve these conflicting data concerning the COR effect on image stabilization three questions were posed which were to be answered by this study

(i) What effect does the frequency of neck torsion have on the magnitude and direction of COR response?

(ii) What effect does the individual s state of arousal or focus of attention have on the COR response?

(iii) Is there a difference between the COR response seen during active as compared with passive neck torsion?

METHODS

Ten subjects (age range 21 to 32 years) 5 male and 5 female took part in the experiments They were not unaware of the nature of the protocol however they did not know the purpose of the experiments

Differential d c recordings between two electrodes (Beckman Miniature Electrodes 650437) placed at the outer canthus of each eye yielded the average horizontal component of eye movement The electro-oculographic (EOG) signal was calibrated while the subject looked in turn at a series of strategically placed light-emitting diodes As a precaution against corneo-retinal potential changes the EOG signal was recalibrated before each trial

Passive body movement

To stimulate the cervico-ocular reflex (COR) in isolation from the optokinetic and vestibulo-ocular reflexes (VOR) the subject stood in the dark with his (or her) head held by means of a dental biteplate clamped to a frame which was rigidly suspended from the ceiling. By biting on the plate the subject could stabilize his head and allow all rotation to take place at the neck

Passive body rotations were performed while the subject stood on a hydraulically driven servo-controlled platform The platform rotated the subject s body about a vertical axis at an amplitude of ±0 4 radians at five selected frequencies −0 025 0 05 0 1 0 2 and 0 4 Hz Individual trials ranged in length from 50 seconds (20 cycles at 0 4 Hz) to 160 (4 cycles at 0 025 Hz) The position of the subject s shoulders and feet (platform) was measured to indicate the absolute amount of neck torsion This was done to ensure that all rotation occurred at the neck and not in the trunk since it has been reported (Biemond & deJong 1969) that pelvic rotation produces nystagmus

One of the following three instructions was given to the subject prior to each trial

(i) Fixate in the dark – The subject was asked to fixate on a central low intensity red light (subtending 0 5 degrees visual angle) and to continue looking at it until it went out After the light was turned off the subject was left in total darkness and was to continue staring at the spot where the light had previously been

(ii) Respond to the tone – The subject was required to listen for the pseudo-random appearance of a soft tone from a speaker located directly behind him This speaker location was chosen to minimize eye movements due to auditory localization (Howard & Templeton 1966). As soon as the subject heard the tone he was to respond by releasing a thumb-activated button mounted in a handle which he held in his hand This terminated the tone This technique served both to arouse the subject and to indicate trends of alertness or drowsiness throughout the duration of the experiment (2–3 hours in a dark room)

(iii) Close eyes and relax – The subject was asked to gently close his eyes to relax, and to attempt to eliminate all thoughts from his mind (that is, not to daydream if possible)

Active body movement

To date all investigations into the COR have involved passive body rotations There is evidence in other neurophysiological systems that responses to stimuli delivered during passive behaviors are non-existent or different from observations made in the presence of active behaviors (Evarts & Tanji 1974 Melvill Jones & Watt, 1971) Therefore it is of interest to study the COR with active as well as passive body movements

A turntable with a low moment of inertia and low frictional resistance was constructed While standing on the turntable with his head fixed by a biteplate the subject was capable of actively rotating his body using only his neck muscles An attempt by the subject to rotate his head to the right resulted in a body rotation to the left since the subject's head was restrained while his body was free to rotate A cyclic attempt by the subject to turn his head alternately to the right and left resulted in active body rotation similar to the passive rotations produced by the servo-driven platform Through the use of the turntable it was therefore possible to produce active neck torsion without labyrinthine stimulation

Experimental procedure

An experiment consisted of three to five trials involving active body rotation and fifteen trials of passive rotation Each of the fifteen trials of passive rotation utilized one of the five stimulus frequencies and one of the three instructions stipulating mental activity Before each experimental trial the EOG signal was calibrated and the subject was given an instruction to follow during the ensuing rotation Rotation then began and lasted for one to three minutes depending on the frequency During this time the subject was standing on the platform in total darkness and was restraining head movement by biting on the bite plate Upon completion of rotation, the subject was allowed to sit and relax if he so chose before beginning the next trial

Data acquisition and analysis

During each experimental trial several channels of data were recorded These included. 1) the horizontal component of eye position 2) the position of the servo-driven platform (during passive rotation) 3) the position of the turntable (during active rotation) 4) the position of the shoulders 5) the state of the EOG calibration lights and 6) the appearance of the audio signal together with the subject's response time A six-channel pen recorder (Gould-Brush 260) was used to monitor the data while the experiments were in progress The data were also recorded on an FM magnetic tape recorder (Honeywell 5600) for subsequent analysis using a PDP 11/50 computer (Digital Equipment Corporation)

Data from the passive experiments were reduced as follows. Using the platform signal as a reference (since it was a pure sinusoid) the computer divided each trial into cycles and then averaged the horizontal eye and shoulder position signals The resulting cycle of averaged shoulder position was considered to be the stimulus and the concomitant cycle of averaged horizontal eye deviation was considered to be the response The coefficients of

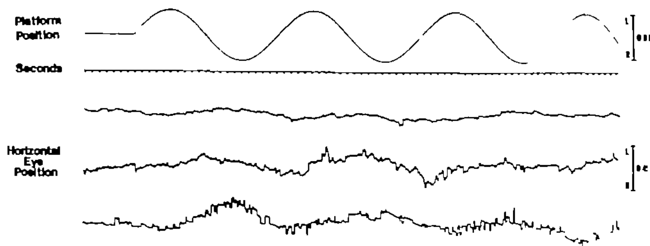


Fig 1 COR responses of 3 subjects illustrating their eye movements before and during body rotation Note the varied character of the eye movements and that vertical calibration for body rotation is twice that for eye move ments (0 4 R=0 4 radians 1 radian=57 degrees).

the first non-d c term i e the fundamental of the Fourier series describing these averaged signals were then found These coefficients i e the amplitude and phase angle of the fun damental frequency component of each signa were used to calculate the gain and phase c the response The gain of the COR respons was found by dividing the amplitude of th output eye deviation by the amplitude of th input shoulder movement The phase angle c the response was a measure of the differenc in phase angle between the two fundamenta and was defined such that a phase angle c zero degrees would indicate an eye movemen in the same direction as the stimulating bod movement A negative phase angle woul signify that the eye response lagged behind th input shoulder motion

A similar type of analysis was performed o the data collected during active body rota tions however due to the fact that the rota tion was actively produced the frequency c rotation varied slightly from cycle to cycle Therefore only those sections of data ex hibiting very small differences in frequenc were averaged and used for further analysis

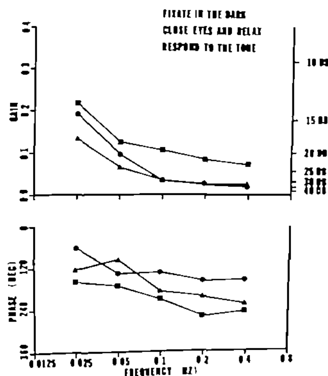


Fig 2 Mean COR response for 10 subjects for each of the three instructions given to the subjects *Uppe* Gain vs frequency of neck torsion where gain is defined as the ratio of slow phase eye movement to amplitude of body rotation *Lower* Phase angle of response (in degrees) vs frequency of neck torsion Gain may also be measured in decibels (dB) from vertical axis on right

RESULTS

Form of cervico-ocular response

The protocol followed in the current study i e body rotation in the dark with the heac held fixed with respect to the environment has demonstrated that cervically produced eye

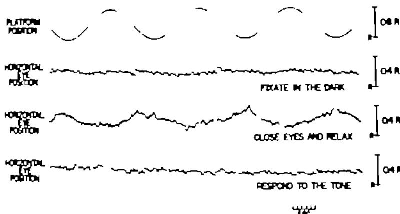


Fig. 3. Comparison between COR responses produced under three sets of instructions. Data shown are from one subject. Note that eye calibration (0.4 radians) is one-half that for the platform position.

movements have certain repeatable characteristics.

Fig. 1 contains a portion of the COR recorded from 3 different subjects. The data were selected to show the general character of neck induced eye movements. As illustrated in the figure, during motionless periods (left side of figure) the subjects exhibited seemingly random eye movements around some central point. Upon the commencement of neck torsion, the baseline began to fluctuate. These fluctuations in the baseline will be referred to as slow phase eye movements (in opposition to the fast phase eye movements or saccades seen in nystagmus). Since typical values for gain and phase angle are given in the following section, it is sufficient at this point to make two observations: first, that the magnitude of the slow phase eye movements are significantly less than the magnitude of the neck movement, and second, that the slow phase eye movement generally occurs in the direction opposite to the direction of platform (or shoulder) movement. That is, a shoulder rotation to the right (clockwise rotation when observing the subject from above) results in a small eye rotation to the left (counter-clockwise when observing the subject from above).

As demonstrated in Fig. 1, slow phase eye movements appear to sum directly with spontaneous eye movements which occur in the absence of neck torsion. The random eye movements which are present before rotation begins continue on throughout the period of neck rotation.

Effects of stimulus frequency

At the lower frequencies of rotation employed cervically induced eye movements are most apparent. As the frequency of neck torsion is increased the magnitude of the resulting eye movement decreases, until at 0.2 Hz there is little eye movement which can be correlated with the neck torsion stimulus.

The decline in response magnitude with increasing frequency is presented in Fig. 2. This figure contains the results of the computer analysis for all 10 subjects under each of the conditions used during the passive experiments. COR response gain is less than 3% at frequencies above 0.1 Hz for the 'Respond to the Tone' and 'Fixate in the Dark' instructions. The largest response is seen at the lowest frequency examined, 0.025 Hz. At this frequency the maximum mean gain observed was approximately 22%.

The phase of the COR response generally ranged between −80° to −240° as seen in Fig. 2. There appears to be a slight increase in phase lag with increasing frequency of rotation, although average phase angle response ranges around −180°. The significance of this fact to ocular stability will be presented in the discussion.

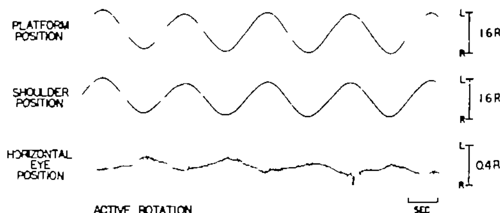


Fig 4 Active rotation in the dark with concomitant eye movements Note that platform and shoulder positions are similar indicating that torsion is taking place at the neck rather than in the trunk region Small high frequency oscillations which occur at peaks of eye mo ement record are caused by electromyographic (EMG) activity sensed by the EOG electrodes The maximum force and therefore EMG activity needed to provide active rotation is produced by neck muscles at the extremes of body position

Effects of subject arousal and focus of attention

The level of arousal and focus of attention of the subjects greatly affect the characteristics of the cervico-ocular reflex Fig 3 shows typical changes in the COR response of a single subject at a single frequency for the three instructions used in the experiments with passive rotations The largest response for all frequencies was produced while the subjects were following the Close Eyes and Relax instruction It is evident that the two instructions which required subject concentration ('Respond to the Tone and Fixate in the Dark) resulted in responses of less magnitude than the instruction which allowed the subjects to relax (see Fig 2)

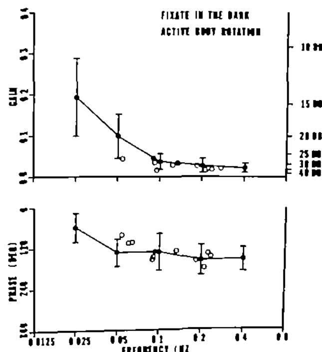


Fig 5 COR responses during active body rotation compared with mean COR response observed during passive body rotation under the instruction Fixate i the Dark Note that for passive rotation COR response is ndicated by a mean and standard deviation at each frequency of rotation averaged for 10 subjects Data points for active rotations are plotted individually

COR response during active rotation

Fig 4 shows a typical COR response during active body rotation Approximately half of the subjects tested were able to produce smooth active body rotations while standing (with their head fixed) on the turntable At the higher frequencies of rotation (around 0 4 Hz) the movements were near sinusoidal When the subjects attempted to produce slower rotations the movements became more triangular and more irregular Since the subjects were unable to produce smooth sinusoidal movements with frequencies below 0 05 Hz the frequency range examined during this part of the study was limited The active movements analysed were between 0 05 and 0 4 Hz.

During preliminary experiments involving

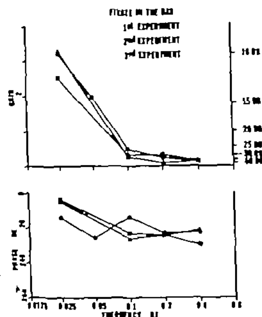


Fig. 6 COR response gain- and phase angle vs. frequency showing intra-subject variability of response. Data are from one subject tested on three different days.

active body rotation large voluntary eye movements were usually present. In an attempt to minimize these unwanted eye movements the subjects were asked to fixate on an imaginary point in space keeping their eyes centered and as motionless as possible while they rotated their bodies in the dark. This command ('Fixate in the Dark') was also used during the passive rotation experiments. The combined results of both the active and passive experiments under this command are shown in Fig. 5. The results indicate a similarity between the COR response under active and passive conditions within the frequency range examined.

Inter- and intra-subject variability of response

In order to establish the repeatability of the data and to examine intra-subject variability in response repeated experiments were performed on 2 of the 10 subjects. Each of these subjects was tested on three occasions. The first and second tests were separated by approximately 6 months the second and third by one week. Fig. 6 contains an example of the results obtained for the instruction Fixate in the Dark.

Intersubject variability can be appreciated by referring to the standard deviation bars in Fig. 5.

DISCUSSION

The most consistent characteristic of the cervico-ocular reflex response i.e. decrease in COR amplitude with increasing frequency of neck torsion confirms the general shape of the results reported by Meiry (1971) although he indicated a larger response amplitude over the entire frequency range than has been observed in this study. This could be due to the fact that he reconstructed the eye signal by removing saccades while these eye signal data are averaged. The amplitude discrepancy cannot be explained fully since Meiry did not describe certain important conditions of his experiments such as the subject's focus of attention and whether the figures represented an average or the largest responses.

Takemori & Suzuki (1971) used only one frequency of rotation (0.25 Hz) in their experiments but their experimental controls and method of data analysis were explained clearly. In one group of experiments their subjects underwent body rotation with their eyes closed. The observed gain of 8% (0.25 Hz) under these conditions is in agreement with the value found during the Close Eyes and Relax command in the present study.

Also the results present the fact that neck induced eye movements almost always occur in a direction approximately opposite to the stimulating body movement. This is of utmost importance when considering the classical concept of the COR's role in image stabilization (see Ocular Stability and COR Function).

Subject level of arousal

Historically the importance of the subject's level of arousal in measurements of oculo-

motor function was realized because of contradictory data reported during vestibular testing Collins & Guedry (1962) discovered that when their subjects were mentally active no decline in nystagmus was evident even during long periods of angular acceleration However during states of mental relaxation nystagmic response was much smaller and occasionally disappeared prior to the end of the stimulus

Similarly Sokolovski (1966) noted that calorically induced nystagmus was consistently greater and more regular in form in subjects who were mentally active when compared with relaxed subjects Therefore it is now generally agreed that drowsiness boredom and fatigue suppress vestibular response while mental activity enhances it

This is in direct contrast to the results found in this study for the cervico-ocular reflex The instruction Close Eyes and Relax required no concentration yet resulted in the largest response (Figs 2 3) The other two instructions required the subject to focus his attention During the instruction Respond to the Tone the subjects were repeatedly reminded of their task by the need to respond to each occurrence of the audible tone This instruction produced the smallest COR responses (Fig 2)

Active rotation

Most of what is known about the cervico-ocular reflex has been derived from passive neck torsion experiments Because of the accumulating body of data on the inhibition or transformation of reflexes when measured during functional behaviors (Evarts & Tanji 1974 Melvill Jones & Watt 1971) the question arose whether active neck rotation might not result in eye movements which differed from the response produced by passive neck torsion The data do not conclusively answer this question since the behavior used was not functional however the movement stimuli were active rather than passive To this extent then the results as presented in Fig 5 show the resemblance between COR data produced by either passive or active body rotation.

Ocular stability and COR function

It has been stated by many investigators that the COR functions to aid in image stabilization upon head movement That is when the head is rotated to the right cervical receptors, stimulated by the resulting neck torsion aid in initiating an eye movement to the left. The two opposing rotations cancel each other and the eye direction in space tends to remain unchanged implying that retinal image slip is reduced by the COR This stated function of the COR will now be reexamined in light of the results of the present study

While the gain of the VOR commonly ranges between 40 and 60% (or as high as 95% under certain conditions (Barr et al 1976)) the highest mean COR gain seen during this study was approximately 22% (although gains for individual runs on occasion ranged as high as 35% and gains for individual cycles of rotation ranged as high as 55%) COR response was found to be largest at very low frequencies of neck torsion—frequencies below those involved in normal head movement If it is assumed that normal human head movements have a frequency content between 0 1 and 5 0 Hz (Melvill Jones & Milsum 1965) then one would expect the COR gain during normal daily movements to be between 2 and 10% Therefore upon a normal head movement of 20° one might expect an eye movement of 2° or less due to cervical stimulation

Consider now the direction of eye movement The experimental responses for all test conditions exhibited a phase angle between −80 to −240°—the average response phase being close to −180 An eye movement which would tend to compensate for a head movement would be in a direction to oppose the head movement—a phase angle of *180°* between the eye and the head movements However in these experiments the head was fixed and neck torsion occurred as a result of body

otation An assumption has been made here that neck receptors will be stimulated equally well by a body rotation (head fixed) as by a head rotation (body fixed) However the direction of rotation would be opposite That is a head rotation to the right (body fixed) would be equivalent to a body rotation to the left (head fixed) Therefore an eye movement which would aid in eye stabilization (as does the VOR) would be in the same direction as the body movement—a phase angle of 0° between the eye and body movements In general then, since eye movement and body movement were 180° out of phase rather than reducing retinal image slip the COR response enhanced it. A similar finding was reported by Gresty (1976) working with rabbits

What then is the function of the cervico-ocular reflex A speculative answer to this question is suggested as follows The vestibolo-ocular reflex provides an image of head acceleration to the oculomotor centers to help in eye fixation during head movement It is proposed that the COR provides an image of body movement to the oculomotor centers The need for separate head image and body image to provide eye fixation becomes apparent during complex movements of head and body For example assume that the head is moving to the left at 5°/sec and the body is also moving to the left but with a velocity of 20°/sec In this case the VOR would produce an eye mo ement toward the right (responding to the 5°/sec head rate) the COR would sense the relative neck torsion to the left (15°/sec rate) and would produce an eye movement also to the right The combined eye movement produced by VOR and COR would therefore tend to reduce retinal image slip

Despite the fact that the summation of VOR and COR effects will not always occur as it does in the example the concept of an eye movement opposed to head motion (VOR) and an eye movement opposed to body movement (COR) is satisfying. Although such a function for the COR is speculative it suggests the path

ACKNOWLEDGMENTS

The authors would like to thank Ruth Christakis for her help in the preparation of the manuscript This research was supported by Grant Numbe 13-P 55518 from the Rehabilitation Services Administration, Department of Health, Education and W lfare Washington, D C

ZUSAMMENFASSUNG

1 Der cervico-okuläre Reflex (COR) beim Menschen wurde gemessen, während die Versuchspersonen (Zahl 10) auf einer Drehplattform standen Der Kopf der Versuchsperson wurde von einer stationären Beißplatte fest gehalten. Die Augenbewegungen wurden als Reaktion der aktiven und passiven Körperrotation um einer vertikalen Achse gemessen

2. Der Verstärkungsfaktor des COR (d h. horizontale Augenbewegung/Schwingungsbreite der Körperrotation) was so stark als 22% bei einer schwachen Frequenz der Körperrotation (0,025 Hz). Bei zunehmender F equenz (z B 0 4 Hz) nahm der Verstärkungsfaktor bis zu % ab

3 Der Phasenwinkel der Augenbewegung lag in den meisten Fallen zwischen 80 Grad und − 40 Grad mit einer durchschnittlichen Reaktion von ungefähr −180 Grad

4 Wahrend der aktiven Rotation war der COR den Reaktionen der passiven Körperrotation ähnlich

5 Das Hauptergebnis ist folgendes Beim normalen wachsenen Menschen unterstützt nicht der COR die Stabilisierung von dem Erscheinungsbild auf der Netzhaut während der passiven oder aktiven Körperrotationen. Eine theoretische Funktion des COR wird vorgelegt und diskutiert.

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Acta Otolaryngol 89: 497–503 1980

VISUAL SUPPRESSION OF CALORIC NYSTAGMUS AND OPTOKINETIC RESPONSES IN CATS

Isao Kato [1] Tadashi Kawasaki Yu Sato[2]
and Yoshio Koike

From the Department of Otolaryngology Yamagata University School of Medicine and the Department of Physiology Toyama Medical and Pharmaceutical University Japan

(Received March 30, 1979)

Abstract Previously it was reported that loss of visual suppression (VS) was accompanied by disturbance of optokinetic nystagmus (OKN) in monkeys with lesions at the flocculus. In the present experiment with cats, OKN was investigated after inflicting lesions on either superior colliculus (SC) or inferior olive (IO), both of which are considered to be the main prefloccular relay nuclei to mediate visual signals to the flocculus. VS of caloric nystagmus was recognized in all the IO-lesioned cats and OKN remained normal except in one cat. After the left SC lesions, loss of VS was evidently revealed and optokinetic stimuli produced directional preponderance to the left with diminished responses to the right in 7 of 9 cats. The present findings suggest that the SC may be an important relay nucleus to the flocculus in conveying visual signals responsible for VOR gain

The flocculus receives primary vestibular signals (Brodal & Høivik 1964) as well as visual signals from the retina (Maekawa & Simpson 1973 Maekawa & Takeda 1975) and sends inhibitory outputs to second-order vestibular neurons which mediate vestibulo-ocular reflexes (VOR) in an excitatory and inhibitory way (Ito et al 1973*a* *b*) On the basis of the neuronal circuitry described above, the flocculus controls VOR gain (eye velocity/head velocity) by utilizing visual signals (Ito et al 1974 Ghelarducci et al 1975 Ito 1976 Lisberger & Fuchs 1978*a* *b*) The gain control of the flocculus might be executed with the aid of oculomotor function which works to stabilize the retinal images while the head is moving In fact a failure in suppressing the slow-phase velocity of caloric nystagmus in man is well correlated with disturbances of the oculomotor functions e g optokinetic response smooth pursuit eye movements and ocular position of gaze (Sato et al in press) Under such conditions as reduced or loss of gain control of VOR optokinetic nystagmus (OKN) involving smooth pursuit eye movements might be also disturbed even in cats The present experiment was concerned with the investigation of OKN in cats with lesions at the superior colliculus (SC) or the inferior olive (IO) both of which are thought to be the prefloccular relay nuclei to mediate visual signals to the flocculus (Maekawa & Simpson 1973 Maekawa & Takeda, 1975)

METHODS

Forty two cats were used in the present experiments. The operative procedures for the animals the techniques of fixing the body of the awake cat those of inducing and recording caloric nystagmus and the method for calculation of visual suppression (VS) in slow-phase velocity were described previously (Kato et al., 1979*a*) Anesthesia, histological identification of electrolytic lesions and the experimental procedures for verifying the IO or the SC were also described in detail in the previous report (Kato et al 1979*b*) Electrolytic lesions of the IO or the SC were focused only on the left side After inflicting lesions on

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William Freedman Ph.D
Arm Research Center
Moss Rehabilitation Hospital
12th Street and Tabor Road
Philadelphia Pennsylvania 19141
USA

Table II *Relationships between VS of caloric nystagmus and lesion site*

Lesion site	Loss of VS	VS
IO		
rostral	0	4
caudal	0	5
SC	7	2

abnormality of OKN OKN-DP to the left alone falls in the lower right corner (shaded area) and OKN DP to the right alone in the upper left corner (shaded area) The other three areas signify bilateral OKN diminution (lower left square) and unidirectional diminution of OK responses combined with OKN DP The lowest and highest nystagmus beats were 33 and 153 which are indicated by horizontal and vertical dotted lines respectively (Fig. 1) Thus the normal range fell in the hexagonal area After the follow-up studies were completed the brain of the animal was perfused with 10% formol-saline solution through the left cardiac ventricle The extent

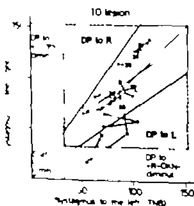


Fig 2 Optokinogram using TNB as measure in the IO-lesioned cats The first open circle in the hexagonal area indicates TNB before IO lesions The subsequent changes of OKN according to time course are indicated with arrows which are interrupted with the cat numbers About week intervenes between the one end of an arrow and the other end for an open circle or square and filled circle The OKN in all the IO-lesioned cat are unaffected and every open square falls within the normal range except for cat no. 15

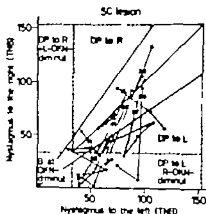


Fig 3 Optokinogram using TNB as parameter OKN in cats with SC lesions tends to show OKN-DP to the left with diminished OK responses to the right except for 2 cats (14 15) belonging to Group I

of lesions was determined by serial sections of paraffin-embedded specimens and reproduced by a projecting device

RESULTS

Of 42 cats investigated 30 survived the electrolytic lesions of the IO or the SC In 25 of the 30 cats, the control test could be performed thoroughly before and after lesions were made In 7 of 25 cats the lesions were found outside the target area. The data for those 7 cats were therefore discarded ultimately Table II summarizes the relationships between VS of caloric nystagmus and the lesion site VS of caloric nystagmus was revealed in all the IO-lesioned cats With the SC lesion VS was not recognized in 7 of 9 cats OKN investigated on these cats is described according to lesion site

IO lesion

In 4 of 9 cats lesions of the IO were found to cover the rostral part, but not the caudal part of the medial accessory olive (MAO) and in the remaining 5 cats lesions completely covered the dorsal cap of the MAO OKN in the IO-lesioned cats were plotted in the optokinogram A normal range of TNB in 9 cats fell in

Table I *Normal limits of each parameter of OKN and normal limits of directional preponderance of OKN in 35 normal cats*

	Parameter OKN		
	TNB	OAL	MES
Mean values	93 1	40.8 (°/sec)	508 1 (μV)
Standard deviation	29 7	13.5	212 4
Normal limits (m+2 S D)	(33–153)	(13–68)	(83–933)
Mean values of DP%	6.5 (%)	5 1 (%)	9 4 (%)
Standard deviation	5 1	3 0	8 6
Normal limits of DP% (m+2 S D)	17	11	27

either IO or SC follow-up studies started from the fourth or the fifth postoperative day onward at about one week s interval Alertness was maintained by an intramuscular injection of amphetamine (0 5 mg/kg) about 30 min prior to the electronystagmographic (ENG) test

OKN was elicited by rotating strips of light projected onto a white screen The cat was placed within about 65 cm distance from an optokinetic (OK) drum (a cylinder 2 m in diameter and 90 cm high) and its subtended visual angle was surrounded with OK screen White strips 5 cm wide were rotated at an angular acceleration of 1 0°/sec² for 60 sec The cats were exposed to two different stimuli applied in both a clockwise and a counter-clockwise direction As indications for abnormal optokinetic responses we chose the total number of beats (TNB) the OK adaptation limit (OAL) and the maximum eye speed (MES) which was calculated from the calibration curves of the apparatus (Honrubia et al 1971) The mean values and normal limits of each parameter in 35 cats before inflicting lesions are shown in Table I An abnormal optokinetic directional preponderance (OKN DP) was established in accordance with the formula (Jongkees & Philipszoon 1964) With L and R representing the left-beating and the right beating OKN responses respectively the percentage OKN DP is

$$DP\% = \frac{L-R}{L+R} \times 100$$

The normal limits obtained from 35 normal cats are presented in Table I Interindividual variation was large Of three parameters TNB was adopted because there was a linear relationship between the square root of the OKN frequency and the logarithm of the velocity of the OK stimulus (Honrubia et al 1971) The normal limits for DP in TNB obtained from 35 cats were set at 17% (Table I) and all individual values for TNB were plotted in the optokinogram (Fig 1) (Mizukoshi et al 1977) This chart represents the normal limits and at the same time gives a survey of conceivable

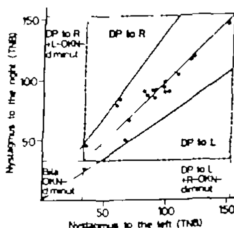


Fig 1 Optokinogram using TNB as a parameter in 35 normal cats Clockwise OK stimulus produces OKN to the left, and counter-clockwise OK stimulus OKN to the right The normal range of TNB is 33–153 nystagmus beats (*hexagonal area*) The shaded areas indicate OKN DP to the right alone and that to the left alone The other three areas include bilateral OKN-diminution (*lower left square*) and unidirectional diminution of OK responses combined with OKN DP



Fig 5 SC lesions. Group II shows large lesions partially invading the pretectum.

OKN also normal In cat 15 VS of caloric nystagmus with the quick-phase to the right was reduced by about 10% in light though normal VS was observed in the left-directed nystagmus This percentage reduction in VS is rather small compared with that for the standard criteria (Kato et al 1979*a*) This phenomenon was transient, however At the 9th postoperative test VS rate had recovered to the normal range OKN at this time showed DP to the left (Fig 3) Histological examination revealed a very small restricted lesion in the SC Seven of 9 cats were found to belong to Group II depending upon the extent of the lesion In these cats loss of VS was persistently recognized After inflicting the lesions the slow-phase velocity of nystagmus with the quick-phase toward the side of the lesions was reduced in light. VS of caloric nystagmus with the quick-phase toward the normal side was lost in light. OKN revealed DP to the left with diminished OK responses to the right as presented in Fig 3

In cats 79 and 38, VS of caloric nystagmus reappeared again at the 12th and 1st postoperative test respectively after making the SC lesion It was then that the IO lesions were inflicted in these cats. VS of caloric nystagmus however was clearly revealed during the subsequent experimental course OKN also remained unchanged OKN of a representative in this series showing loss of VS of caloric nystagmus is presented in Fig. 4 The upper trace in A–F is an ordinary curve (time constant 1.5 sec) and the second trace in each series a derived curve (time constant 0 03 sec). The bottom trace in E and F is a drum speed of OK stimuli recorded with a photocell Upward and downward arrows in each series mark the moment of onset and end of OK stimulus respectively A C and E represent OKN to the left with OK strips rotated to the right. B D and F show OKN to the right OKN before the lesions is shown in A and B OKN after the lesions in C, D E, and F At the 9th postoperative test the left directed OKN showed a slight decrease in response (C) while OKN to the right diminished by 56% (D) At the 26th test after the SC lesion the right-directed OKN is still reduced by 26% (F) though the OKN toward the left side was nearly normal (E) In this case OKN showed DP to the left with diminished OK responses to the right after the lesions, and tended to recover to the normal range with the lapse of time (Fig. 3) In this

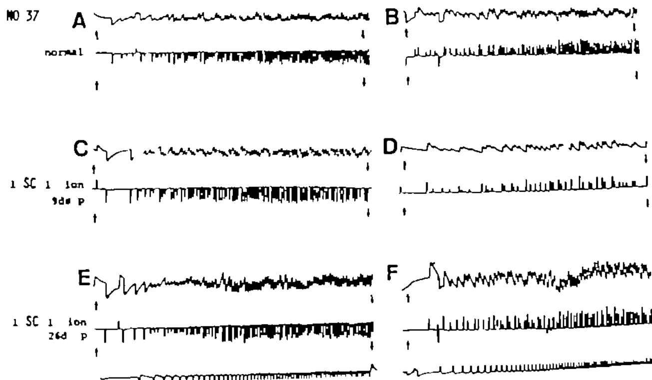


Fig 4 Effects of left SC lesions upon OKN in a cat representative of Group II A B normal OKN before SC lesions At the 9th test, left-directed OKN shows a slight decrease in response (C) and OKN to the right is reduced by 56% (D) At the 26th test after lesioning right-directed OKN is still reduced by about 76% (F) though OKN toward the left side is near normal (E). Optokinogram in the cat 37 shows OKN-DP to the left with diminished OK responses to the right (Fig. 3)

the hexagonal area (Fig 2) The first open circle in the hexagonal area indicates TNB be fore inflicting lesions The next open square represents TNB recorded about a week later after the IO had been lesioned The subsequent changes of OKN according its time course are indicated with arrows though these are interrupted with the cat numbers The filled circles represent SC lesions which were inflicted in the cat 35 and 39 About a week intervened between the one end of an arrow and the other end for an open circle or square and a filled circle Symbols in Fig 3 signify the same meanings as above The OKN in all the IO-lesioned cats were unaffected and every open square fell in the normal range except for cat no 35 In cats 35 and 39 the SC was lesioned subsequently on the 12th and 14th day from the IO lesions In these two cats VS of caloric nystagmus with the quick phase to the right was lost after the SC had been lesioned OKN revealed DP to the left with diminished OK responses to the right in the 35 and DP to the left in cat 39 (Fig ?)

SC lesion

Cats with SC lesions were classified into the following two groups depending upon the ex tent of the lesions Group I with lesions in the very small restricted area and Group II with lesions encroaching in almost all the anterior half of the SC and extending rostrally to a part of the pretectum In all the SC-lesioned cats the eye movements were normal and the pupils showed normal response to light When TNB was used as a parameter the optokinogram as illustrated in Fig 3 was obtained in all the SC-lesioned cats The overall tendency of OKN after making the lesion in the SC showed DP to the left with diminished OK responses to the right except for 2 cats (nos 14 15) belonging to Group I In cat no 14 VS of caloric nystagmus was clearly observed throughout the whole experimental course and

urde an 42 Katzen OKN nach Läsion der unteren Olive und des Colliculus superior wodurch der Flocculus das visuelle Signal empfängt, untersucht. Nach Läsion der unteren Olive urde die visuell ausgelöste Suppression des kalorischen Ny tagmus immer noch beobachtet und OKN abgesehen von einer Katze auch normal ausge lost Nach Colliculusläsion wurde der Effekt des Lichts auf den kalorischen Nystagmus an 7 von 9 Katzen nicht beobachtet und OKN in diesen Katzen zeigte das Richtungsuberwiegen nach links und die Verminderung oder den Ausfall nach rechts Von diesem Resultat darf man daher ohl sagen, daß Colliculus superior als praflocculärer Kern eine wichtige Rolle spielt.

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Dr I Kato
Department of Otolaryngology
Yamagata University School of Medicine
Zao-Iida
990-23 Yamagata City
Japan

cat loss of VS lasted for 75 days Fig 5 showed lesions in the SC Rostrally placed lesions in the SC invaded the pretectum partially

DISCUSSION

The flocculus exercises gain control of VOR by using visual information which has the effect of improving the stabilization of retinal images during head movement (Ito et al 1974) When the fixed slit light is presented to an eye with the rabbit mounted on the turntable the horizontal movement of that eye during head rotation is enhanced in comparison with that in darkness In contrast, the eye movement is reduced by a slit light moving in phase with the turnable This gain control is effectively abolished when the flocculus is extirpated (Ito et al 1974)

In the rhesus monkey caloric nystagmus is suppressed in light as compared with that in total darkness (Takemori & Cohen 1974*a*) This visually induced suppression of caloric nystagmus also occurs in the direction of stabilizing retinal images of the visual surroundings In fact visual suppression of caloric nystagmus is lost after the flocculus is lesioned (Takemori & Cohen 1974*b*) Furthermore it is noticeable that OKN is also disturbed in monkeys with lesions in the flocculus (Takemori & Cohen 1974*b*) Two possible explanations come to mind for the release of visual suppression of caloric nystagmus the one is a disturbance of the gain control system of VOR exerted directly by the flocculus and the other is the disturbance of the primary oculomotor system such as gaze holding smooth pursuit eye movements and optokinetic responses The latter is suggested by the present experiment showing that in cats with lesioned SC loss of VS is recognized and OKN disturbance is evident

The dorsal cap of the MAO could be one of the main relay nuclei to mediate visual signals to the flocculus (Alley et al 1975 Takeda & Maekawa 1976) In our previous experiment electrolytic lesions were placed at various antero-posterior levels of the IO (Kato et al 1979*b*) In particular the dorsal cap of the MAO was confirmed by responses to both light-guided and antidromic field potentials with a bipolar stimulating electrode inserted into the contralateral flocculus Electrolytic lesions of the dorsal cap resulted in complete loss of a late component of the field potentials from the flocculus Even in the cats prepared in this fashion VS of caloric nystagmus was clearly demonstrated (Kato et al 1979*b*). Furthermore in the present experiment, the IO lesions had no effect upon OKN These findings are strongly supported by many reports suggesting that the IO is not related to the temporary modification of the VOR by visual stimuli (Ito 1976 Kawasaki et al 1978 Kato et al 1979*b*) An electrical stimulation of the SC evoked pronounced mossy fiber responses in the flocculus even after severance of the accessory optic tract Furthermore the early component of field potentials (Maekawa & Takeda 1975) was reduced after SC lesions (Kawasaki et al 1978) On the basis of the present findings showing that the loss of VS and disturbance of OKN are clearly demonstrable in SC lesioned cats it may be assumed that the SC is an important relay nucleus to the flocculus for conveying the visual signals responsible for VOR gain

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Prof N Maruyama, Dept of Neurophysiology Brain Research Institute Niigata University for his useful advice The authors also wish to acknowledge the technical assistance of Mr S Tamura and Mr N Taga

This investigation was partially supported by grant no 457419 of the Ministry of Education of Japan

ZUSAMMENFASSUNG

Der Verlust der visuell ausgelösten Suppression des kalorischen Nystagmus wurde nach Läsion des Flocculus in den Affen demonstriert, dann wurde optokinetischer Nystagmus (OKN) auch gestört In diesem Experiment

Fig. 1 Changing the low-pass filters from 15 to 30 and to 70 Hz does not significantly influence the velocity of the saccade as calculated from the recordings

15 Hz (Mingograph M 81 Siemens-Elema Stockholm Sweden) served as a recorder

Before the selection of the filter was made the rise time of the amplifier was tested by using square waves and ramp signals corre sponding to different angular velocities of the eyes. When filters of 15 30 70 or 700 Hz were used, the amplitude the shape and the duration of these "electrical saccades" did not change noticeably when signals with a rise time of 50 ms or longer were applied To exclude high frequency noise we could therefore use the 15 Hz filter without any distortions of the signal (see also Fig. 1)

The paper was fed at 100 mm s to secure the accuracy in the analysis of recordings for events occurring within 10 ms (1 mm) In the recording an angular deviation of the eyes of 1 corresponded to 1 mm

The patient was sitting comfortably in a chair The head was mechanically supported and the position of the head adjusted (eye ear axis horizontal) for each subject before any measurements was undertaken Light diodes were placed 120 cm in front of the subject at angles of 5° 10° 20° and 30° to the right as well as to the left of the midline Calibration was made with the subject looking alternately at the diodes which were 20° and 60° apart

Saccades The tests were performed in darkness The targets consisting of light diodes of different colours were therefore easy to distinguish At least 10 saccades at each of the amplitudes 5° 10° 20° 30° 40° and 60° were performed. The light diodes were then switched off and the subject was asked to look first with his eyes open and then to try to look also with his eyes closed at the diodes 10° apart 20° apart, and finally at those 60° apart.

Caloric test A routine caloric test (Hen-

Fig. 2 The peak velocities of rapid eye movement are measured and classified according to amplitude of the eye movement The tangent at peak velocity is visually determined for the recording and is calculated (p1 A/T) by using the amplitude (A) and duration (T) at peak velocity (p1°).

Acta Otolaryngol 89 504–512 1980

VELOCITY PATTERNS OF RAPID EYE MOVEMENTS

N G Henriksson I Pyykkö L Schalén and C Wennmo

From the Department of Otorhinolaryngology University Hospital Lund Sweden

(Received May 3 1979)

Abstract The peak velocities of saccades and fast phases of nystagmus were examined and compared in 20 healthy subjects. The peak velocities of both types of eye movements increased with increase of amplitudes The saccades were found to be fastest in light slower in darkness and slowest behind closed eyelids The peak velocities of the quick phases of optokinetic and of vestibular nystagmus were found to be the same Fast phases of optovestibular (optic as well as vestibular stimulation) nystagmus produced significantly higher peak velocities than the two others At the same amplitude and during the same visual conditions the saccades were significantly faster than any type of fast components of nystagmus. The difference in velocity between voluntary and reflexive eye movements is possibly related to differences in antagonistic activity during these eye movements, but also to specific synaptic events during the voluntary action

There are two main groups of rapid eye movements the saccades and the fast phases of nystagmus Whereas the saccades can be triggered voluntarily or spontaneously by peripheral visual stimuli the fast phases of vestibular and of optokinetic nystagmus are triggered reflexively secondary to the slow phase of nystagmus

All rapid eye movements seem to utilize the same pontine neuronal network located in the parapontine reticular formation (Cohen & Feldman 1968 Cohen & Komatsuzaki 1972 Cohen et al 1973 Henn & Cohen 1975) where the position of the eyes is adjusted to the position of the target (Robinson 1975) A similar firing pattern is thus found in these neurons during saccades as well as during fast phases of nystagmus (Keller 1974 Henn & Cohen 1975)

This would support findings by several authors (Dichgans et al 1969 Nauck et al 1969 Ron et al 1972) who neither in man nor in intact alert monkey found differences between saccadic velocity and velocity of fast phases of nystagmus

As these findings were in contrast to our observations the present study of the velocity of the two types of rapid eye movements was undertaken

The aim of this paper will be to determine the velocities of the fast phases of nystagmus of 20 normal subjects exposed to rotation to caloric stimulation as well as to optokinetic stimuli for comparison with the velocity of the voluntary saccades at corresponding amplitudes

MATERIAL AND METHODS

The material consisted of 20 volunteers with normal hearing and vision They had no history of neurological disease and a routine otoneurological examination revealed free eye movements Ten were males and 10 females and their mean age was 48 years with a range of 23 to 72 years An electro-oculographic (EOG) technique with commercial electrodes and electrode jelly (Medicotest®) was used

In the horizontal plane the mean of the movements of the right and left eye was recorded by the use of conventional electrodes horizontally disposed while the recording of vertical eye movements was made for each eye separately A DC-coupled ink writer with an upper cut-off frequency of

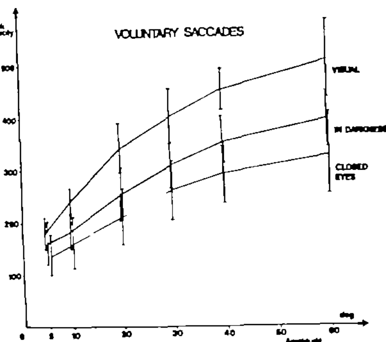


Fig. 3 Mean peak velocities with standard deviations of voluntary saccades when performed in visual conditions, in darkness and in darkness behind closed eyelids at different amplitudes.

At 20° amplitudes the mean peak velocity of the saccades in light was about 340° s in darkness about 250° s and behind closed eyes about 210° s (Fig. 3) From these values the peak velocities increased with increasing amplitudes of the saccades and at amplitudes of 60° were 500° 400° and 320° s respectively (Fig. 3)

Saccades were characterized by highly variable velocities when performed without visual control Sometimes efforts to produce saccadic eye movements with closed eyes resulted in recordings appearing more as slow deviations of the eyes than as fast saccadic eye movements (Fig. 4) In darkness, over shooting saccades were very common especially at the amplitudes of 10° and 20°

There was further a considerable inter individual variation in saccadic velocity Since this study was focussed on the mean values of a whole group of subjects examined in standardized visual conditions no further interest was paid to either inter or intraindividual variations.

We found no difference in the peak velocities of saccades between different age groups (Fig. 5) Young subjects had no higher saccadic velocity than older ones in fact a subject aged 72 had faster saccades than another subject aged 22.

The rapid return eye movements following the pursuit eye movements were analyzed in five subjects. These rapid eye movements had the same amplitude-velocity relationship as visual saccades Hence we considered these movements identical to the saccadic ones.

Fast phases of nystagmus We found no difference in the peak velocities of the fast phases of caloric nystagmus, whether induced by irrigation of the right or of the left ear by cold or by warm water These peak velocities were as were the velocities of the saccades strongly dependent upon the amplitude of nystagmus as shown in Fig. 6 Moreover the fast phases of nystagmus were equal whether caused by rotation or by caloric stimulation The peak velocities of the fast phases of nystagmus induced by rotation at amplitudes of 20° were about

Table I *Mean peak velocities with spreads of voluntary saccades at amplitudes of 20° and of 60° in two groups of subjects tested in two laboratories*

	No	Age (years) mean and range	Laboratory	Peak velocity of saccades at 20° mean ± S D	Peak velocity of saccades at 60° mean ± S D
Group A	20	48 4 (23–72)	I	336° s ± 42° s	495° s ± 85° s^{-1}
			II	327° s ± 61 s	480° s ± 96° s
Group B	12	45 0 (18–70)	II	316° s ± 67° s	465° s ± 96° s

riksson et al 1972) was conducted in darkness with the subject s eyes open

Rotation test The rotation test was performed in complete darkness by acceleration of the subject within one second to a constant velocity of 120° s^{-1} After one minute of rotation the chair was brought to a standstill within one second The perrotatory as well as the postrotatory nystagmus was recorded with the eyes open

Optokinetic test The optokinetic test was performed with the subject inside a striped drum rotating around the patient The drum was accelerated for 10 seconds to a constant velocity of 90° s^{-1} and the calculations were made at velocities of the drum at 90° s^{-1} as well as somewhat below this value

Optovestibular test (optokinetic + rotation stimulus) In this test the subject was rotated inside the optokinetic drum with his eyes open Using this technique eye movements were recorded resulting from a combination of vestibular and optokinetic stimuli The physical properties of the acceleration in this combined test were the same as those in the rotation test in darkness

Evaluation of recordings The peak velocities were measured by estimating the tangent for rapid eye movements this usually occurred at the beginning of the eye movement The saccades and the fast phases of nystagmus were classified into different groups according to their amplitudes (Fig 2) The means and standard deviations of the velocities of the eyes in the saccades and in the fast phases of the various types of nystagmus were determined for each individual and for each amplitude (10° 20° 30° 40° and 60°)

To assess the stability of the mean saccadic velocity within the same group of normals this velocity was determined on two different occasions with an interval of about one hour Two different sets of recording devices in two different laboratories were used

To study the variation between two different groups of normals another group of 12 subjects was examined with a similar age distribution as the subjects in the main study (Table I)

RESULTS

Saccades The mean peak velocities of the saccades at amplitudes of 20° and 60° for the same group of subjects in two different laboratories are provided in Table I Despite the fact that the recordings were made in different laboratories with an interval of one h the mean peak velocities were very close Also when two separate groups of subjects were examined the mean peak velocities were quite similar (Table I)

The amplitude—peak velocity relationship of saccades light in darkness and with closed eyes is shown in Fig 3 Saccade peak velocity was dependent not only upon the amplitude but also on the visual condition with a given visual condition an increase in amplitude produced an increase in the peak velocity the function being curvilinear

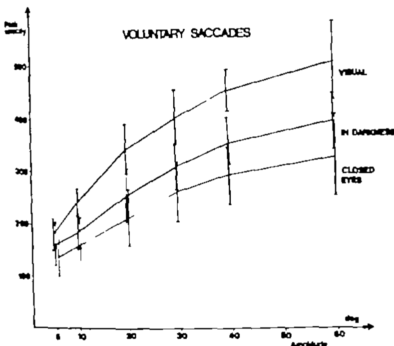


Fig. 3. Mean peak velocities with standard deviations of voluntary saccades when performed in visual conditions, in darkness and in darkness behind closed eyelids at different amplitudes.

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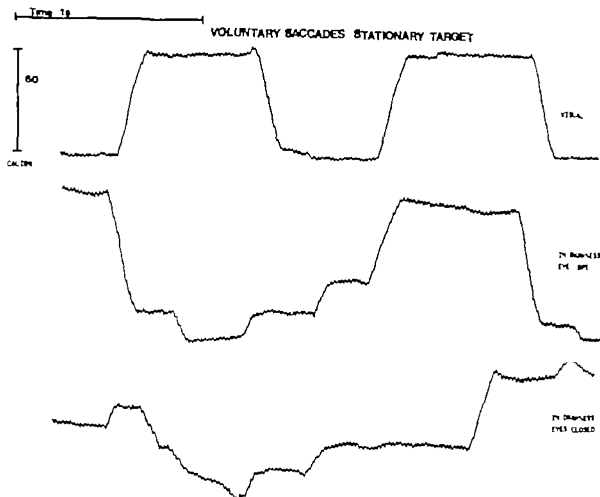


Fig 4 A typical recording showing voluntary saccades when performed in visual conditions in darkness eyes open and in darkness behind closed eyelids

215° s^{-1} Velocities at higher amplitudes could not be determined as such nystagmus is rare in adults (Tibbling 1969)

The *amplitudes of OKN (optokinetic nystagmus)* were generally smaller usually below 20° By increasing the velocity of the stimulus the amplitude of nystagmus tended to decrease Hence for optokinetic nystagmus we obtained only a few measuring points for higher amplitudes than 20° The amplitude velocity relationship for the fast phases of OKN is also presented in Fig 6 For the measurable amplitudes the OKN fast phase velocities closely followed the corresponding curves for the fast phase of vestibular nystagmus

By rotating the subjects within the optokinetic drum with the subject s eyes open and in light (combining visual and vestibular stimuli) the peak velocity of the fast phase of nystagmus increased (Fig. 6) The *peak velocity of the fast phases of the combined* nystagmus about 260° s^{-1} and the amplitude of 20° against 215° s at the same amplitude for pure vestibular or pure optokinetic nystagmus

Comparison of velocities of saccades with fast phases of nystagmus Fig 7 shows a summarising diagram of the peak velocities of saccades and of fast phases of nystagmus with eyes open in light or in darkness With the same visual condition and at the same amplitude the saccades were always faster than the fast phases of any type of nystagmus here examined The difference was more pronounced at greater amplitudes.

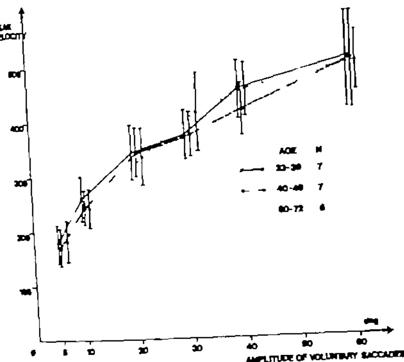


Fig. 5 Mean peak velocities with standard deviations of visual voluntary saccades in three different age groups at different amplitudes.

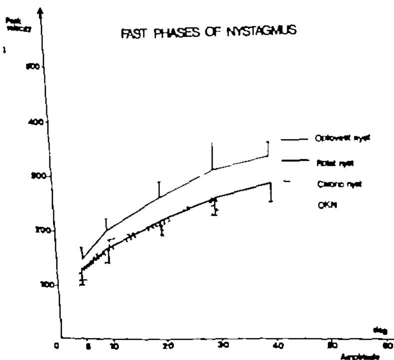


Fig. 6 Mean peak velocities and standard deviations of fast phases of optovestibular nystagmus (—), of rotatory nystagmus (—) of caloric nystagmus ( ) and of optokinetic nystagmus ( ). The spreads are presented only in direction

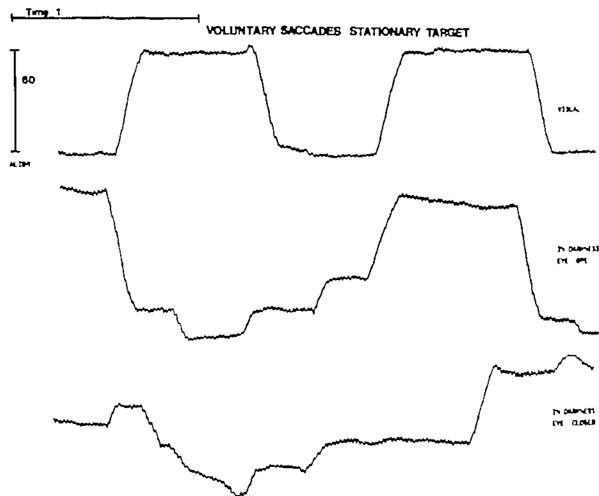


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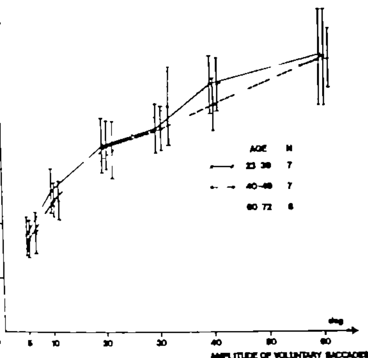


Fig. 5. Mean peak velocities with standard deviations of visual voluntary saccades in three different age groups at different amplitudes.

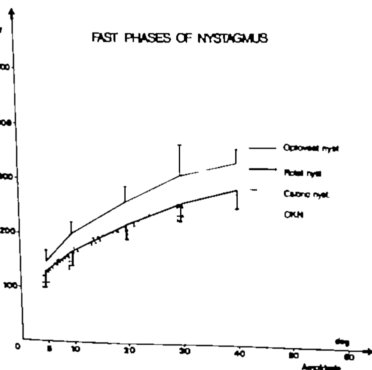


Fig. 6. Mean peak velocities and standard deviations of fast phases of optovestibular nystagmus (—) of rotatory nystagmus (—), of caloric nystagmus ( ) and of optokinetic nystagmus ( ). The spreads are presented only in direction

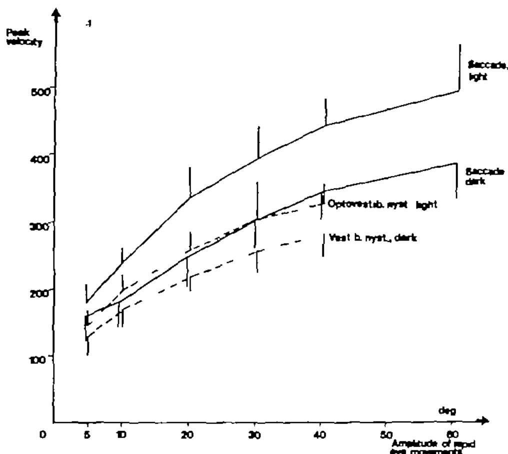


Fig 7 A summarizing diagram showing the difference between velocities of saccades in light and of fast phases of optovestibular nystagmus. The difference is also shown between the velocities of the saccades in dark and the velocities of fast phases of rotatory nystagmus (vest. nyst. dark) The parameters are as in Figs. 3 and 4

DISCUSSION

It must be stressed that during the saccade the velocity is not constant The peak velocity is usually reached somewhat before the middle of the saccades after which the velocity gradually declined (Westheimer 1954 Collins 1975) Hence the saccades represent typical ballistic movements of the eyes (Robinson 1964) Our reason for measuring peak velocity instead of duration or mean velocity of saccades was due to an observation by Keller (1974) who found a highly synchronous firing pattern within the pontine neurons during peak velocities We have therefore assumed that peak velocity should be more sensitive to disturbances in clinical connection than other parameters of the saccades (Baloh et al 1975)

The difference in velocity between saccades and fast phases of nystagmus has not been reported earlier The complexity in oculomotor mechanism makes the observation difficult to explain Thus the cortical mechanism involved in visual saccades is not yet fully understood Single unit recordings have shown activity during saccades in the neurons of Brodmann s area 8 (Bizzi 1968 Bizzi & Schiller 1970) area 7 (Straschill & Schick 1974 Mountcastle et al 1975 Lynch et al 1977) but also in areas 17 18 19 (Hubel & Wiesel 1965 Orban & Callens 1977*a* *b*) The importance of these centres is further supported by lesion studies (Pasik & Pasik 1964 Latto & Cowey 1971 1972)

The efferent pathways from these three cortical fields descend to neurons in the pontine reticular formation (Astruc 1971) Through other pathways the same pontine neuronal network is also activated during the fast phases of vestibular and optokinetic nystagmus From there oculomotor impulses are channeled to the oculomotor nuclei through final pathways common to all different types of rapid eye movements (Henn & Cohen 1975) This conception of a final pathway common to the oculomotor system has sup-

ported the idea that all types of rapid eye movements should be equally rapid. Our observations to the contrary may have some support in previous literature also.

Thus the normal contraction of the antagonistic muscles present only during the fast phase of nystagmus (Shimazu 1972) might explain a difference in velocity between fast phases and saccades. Further the "slow" saccadic velocities noticed e.g. in Huntington's chorea, has been believed to be due to lack of proper inhibition of antagonistic muscles (Starr 1967).

One other factor which seems to be of importance is that saccades are initiated voluntarily but fast phases of nystagmus are released automatically and secondarily to slow phases. During voluntary saccades the subject is alert and active performing a task while during fast phases of nystagmus the subject is passive. The higher velocity of the saccades might therefore at least in part be explained by a more powerful excitation of pontine neurons caused by volition or related to alertness caused by this volition.

ZUSAMMENFASSUNG

Willkürliche schnelle Augenbewegungen (Saccaden) und reflexbedingte schnelle Augenbewegungen (schnelle Nystagmusphasen) wurden bei 20 gesunden Personen untersucht und miteinander verglichen. Die Geschwindigkeiten beider Augenbewegungstypen stiegen mit einer Zunahme der Amplituden an. Es wurde gefunden, daß die willkürlichen Augenbewegungen, die Saccaden, im Licht am schnellsten, langsamer in Dunkelheit und am langsamsten hinter geschlossenen Augenlidern sind. Während die Geschwindigkeiten der schnellen Phasen im optokinetischen und vestibulären Nystagmus identisch waren, erreichten die schnellen Phasen von optovestibulären Nystagmen (sowohl optische als auch vestibuläre Stimulation) signifikant höhere Geschwindigkeiten als beide anderen Typen. Bei gleicher Amplitude und unter gleichen visuellen Bedingungen waren die Geschwindigkeiten der willkürlichen schnellen Augenbewegungen (der Saccaden) signifikant höher als die schnellen Komponenten reflexbedingter Augenbewegungen.

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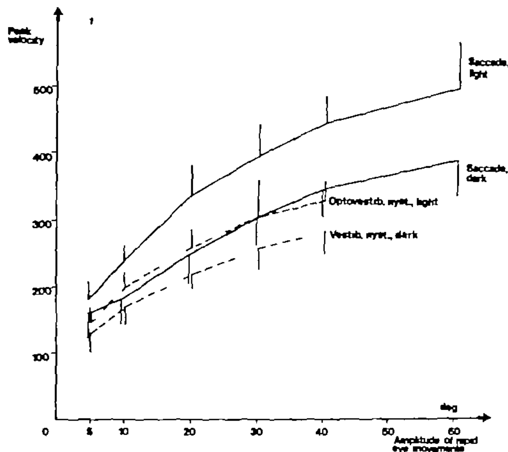


Fig 7 A summarizin diagram showing the ence between velociti saccades in light and phases of optovestibu nystagmus. The diffe is also shown betwee velocities of the sacca dark and the velocitie fast phases of rotatory nystagmus (vest. nyst dark). The parameter are as in Figs. 3 and 4

DISCUSSION

It must be stressed that during the saccade the velocity is not constant The peak velocity is usually reached somewhat before the middle of the saccades after which the velocity gradually declined (Westheimer 1954 Collins 1975) Hence the saccades represent typical ballistic movements of the eyes (Robinson 1964) Our reason for measuring peak velocity instead of duration or mean velocity of saccades was due to an observation by Keller (1974) who found a highly synchronous firing pattern within the pontine neurons during peak velocities We have therefore assumed that peak velocity should be more sensitive to disturbances in clinical connection than other parameters of the saccades (Baloh et al 1975)

The difference in velocity between saccades and fast phases of nystagmus has not been reported earlier The complexity in oculomotor mechanism makes the observation difficult to explain Thus the cortical mechanism involved in visual saccades i yet fully understood Single unit recor have shown activity during saccades in neurons of Brodmann s area 8 (Bizzi Bizzi & Schiller 1970) area 7 (Stras & Schick 1974 Mountcastle et al Lynch et al 1977) but also in areas 17 19 (Hubel & Wiesel 1965 Orban & Call 1977*a* *b*) The importance of these cer is further supported by lesion studies (P & Pasik 1964 Latto & Cowey 1971 197

The efferent pathways from these t cortical fields descend to neurons in pontine reticular formation (Astruc 19 Through other pathways the same pon neuronal network is also activated during fast phases of vestibular and optokinetic r tagmus From there oculomotor impulses channeled to the oculomotor nuclei thro final pathways common to all different ty of rapid eye movements (Henn & Coh 1975) This conception of a final pathw common to the oculomotor system has s

Acta Otolaryngol 89 513–523 1980

THE MECHANISM OF PHYSIOLOGICAL HEIGHT VERTIGO

I *Theoretical Approach and Psychophysics*

Thomas Brandt Friedrich Arnold Willem Bles and Theo S Kapteyn[1]

From the Neurological Clinic with Clinical Neurophysiology Krupp Hospital Essen Federal Republic of Germany and the E.N.T Department Free University Hospital Amsterdam The Netherlands

(Received April 23 1979)

Abstract A theory is presented supporting a geometrical explanation of physiological height vertigo as a distance vertigo created by visual destabilization of posture when the distance between the observer and visible stationary contrasts becomes critically large. Though height vertigo is generally regarded as a psychopathological process, we hypothesize that it might instead result from an inter sensory mismatch when visual information is at variance with vestibular and proprioceptive inputs. Psychophysical experiments confirming the hypothesis revealed that 1) height vertigo is clearly related to body position, being the greatest in the upright stance 2) it is the eye-object distance rather than the direction of gaze which is critical; 3) there is a saturation of height vertigo magnitude Subjective vertigo increases with increasing altitude only below 20 metres. Physiological distance vertigo must be distinguished from psychological acrophobia Its postural consequences may be ameliorated by strategies derived from knowledge of its mechanism such as providing nearby stationary contrasts in the peripheral visual field.

Height vertigo a visually induced syndrome commonly experienced on top of high struc tures is manifested by a subjective instability of posture and locomotion coupled with a fear of falling and vegetative symptoms It is similar to motion sickness in that both are acute physiological symptoms which spontaneously remit after termination of the inducing stimulus situation. Also in analogy to motion sick ness habituation to height vertigo may occur through repeated exposure such as observed in steeplejacks roof workers and tight rope artists, who achieve a remarkable degree of postural balance with seeming insensitivity to height

Height vertigo generally has been attributed to psychopathological processes such as neurotic acrophobia or neurasthenic dizziness We believe that height vertigo does not simply represent an unpleasant visual epiphenomenon but rather is a meaningful warning signal to the body for withdrawal from stimulus situations which cannot be adequately perceived in terms of space constancy necessary for postural control. We are postulating a physiological mechanism for height vertigo separate and distinct from purely cognitive or psychological factors Psychophysical data are presented in this paper and posturographic data in a subsequent study (paper II Bles et al 1979) which support a simple geometrical explanation for physiological height vertigo as being a distance vertigo

We reasoned that a visual destabilization of postural balance would induce height vertigo when the distance between the observer and visible stationary contrasts became critically large A brief review of the visual contribution to balance in man will be presented as a necessary prelude to assist in understanding our hypothesis and experimental design.

Optimal balance requires the continuous evaluation of the reafferent sensory consequences of self-generated body movements There are three main loops which control posture 1) vestibular 2) somatosensory (proprioceptive) and 3) visual Each control loop has its own 'frequency range' of optimal func

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N G Henriksson M.D
E.N T Department
Hospital of Lund
S-221 85 Lund
Sweden

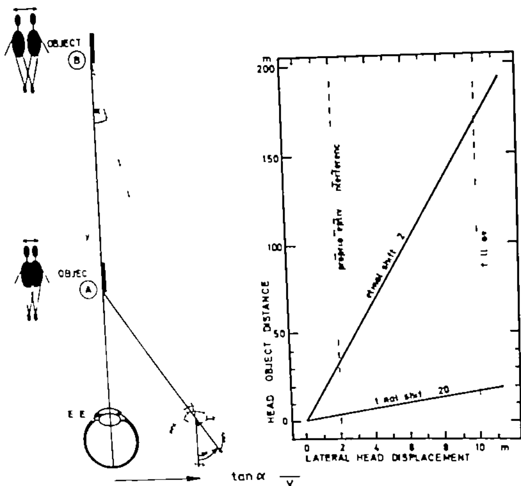


Fig. 1. Geometrical analysis indicates that in order to be visually detected, body sway must increase with increasing distance between the eyes and the nearest stationary contrasts. Angular displacements α on the retina, caused by lateral head displacement, are smaller the greater the distance to the object. Diagram shows relationship between head-object distance y and lateral head displacement, for given retinal displacement thresholds of either 2 or 20 minutes of arc. However, because postural regulation involves multiloop control, additional proprioceptive cues may alter sway amplitudes as well.

1955) a normal lateral head sway of 2 cm would be subthreshold at a distance of about 3 m. This should lead to a perceptual conflict as the vestibular and somatosensory receptors sense a body shift which the visual system cannot detect. The conflict might be resolved by increasing the postural sway and thereby reactivating visual control. For a simple loop control a relationship between distance and sway amplitude could be expected (tan α = /y) with the gain dependent on the retinal movement detection threshold. However with the lower limbs close together at free stance one falls over at a head sway more than 10 cm. Thus at an eye–object distance of 15–20 m a maximal body sway' would produce retinal angular deviations (α) less than 20 min of arc according to the trigonometrical model. Since we are dealing with a multiloop control of postural balance it can be assumed that with increasing sway amplitudes the particular sensory weight of the somatosensory and vestibular afferences becomes greater increasing their contribution to pos-

tion With some overlap among the ranges they cover the entire range encountered in daily life

Vision plays a major role in postural stabilization It attenuates self generated sway by 50–100% (Edwards 1946 Travis 1945) and its effect on postural stability accounts for the significance of the Romberg test in clinical examinations (Romberg 1846) The role of vision is particularly apparent in patients with diseases involving the proprioceptive joint and muscle afferents as in tabes dorsalis (Duchenne 1958 Frenkel 1907) or in patients suffering from postconcussional dizziness (De Wit & Bles 1975) and is also important to a lesser degree in patients with cerebellar (Dichgans et al 1976) or vestibular lesions (Barré 1949 De Haan 1959 De Wit 1973) Under experimental conditions vision may have a strong destabilizing effect on posture particularly when visually perceived motion does not adequately correspond with the actual body shift sensed by the vestibular and somatosensory systems Observations of an optokinetically induced body sway have been reported in the older literature by Fischer & Kornmüller (1930) Extensive experiments on that phenomenon have been performed with movements of an artificial surround as circular motion about the vertical Z axis (Kapteyn & Bles 1977) roll motion about the line of sight (Dichgans et al 1976) a sinusoidally tilting room (Bles et al 1977) and linear motion of the seen environment (Lee & Aronson 1974 Berthoz et al 1975) In these instances there was an optokinetically induced perception of apparent self motion opposite in direction to the pattern motion The postural reflexes compensate for this subjective body shift resulting in a measurable body tilt in the direction of the motion pattern These optokinetically induced postural reactions require stimulation of the entire visual field or substantial portions of the periphery This corresponds to the stimulus characteristics of visually induced vections Dynamic spatial orientation and postural control rely mainly on information from the retinal periphery (Brandt et al 1973 Dichgans & Brandt 1978)

Studies on children revealed that the visual contribution to postural stabilization participates rather late after the acquisition of upright stance and gait (Brandt et al 1976) Furthermore the calibration of the three main loops (vestibular somatosensory and visual) occurs sequentially is mutually interactive and remains plastic throughout life In order to maintain postural stability in the upright position afferent signals must be generated as an input for compensation of natural fore–aft and lateral body sways The measurable sway path of the centre of gravity is greater in the fore aft than in the lateral direction Fore–aft sway is comparable to the motion of an inverted pendulum as a first approximation (Nashner 1970) whereas lateral sway is horizontal over several centimetres (parallel shift) because of the mechanical joint relationship of the lower limbs pelvis and vertebral column (Kapteyn, 1973) A lateral sway causes a shift of the visible surround on the retina when the eyes are stationary The importance of retinal image motion in stabilizing posture is supported by experiments utilizing stroboscopic surround illumination Visual stabilization was practically absent at strobe frequencies up to 6 Hz (Amblard & Cremieux 1976)

Hypothesis

Simple geometrical analysis indicates that body sway must increase with increasing distance between the eyes and the nearest stationary contrasts in order to be visually detected (Fig 1) Angular displacement α on the retina is smaller the greater the distance Y to the object becomes Since the natural lateral head sway is about 2 cm in amplitude (Kapteyn 1973) an important determination would be the specific eye–object distance at which this sway amplitude could not be visually detected Since an angular displacement of the visual scene of 20 min of arc is necessary for detection by the paracentral and peripheral parts of the retina (Aubert 1886 Leibowitz

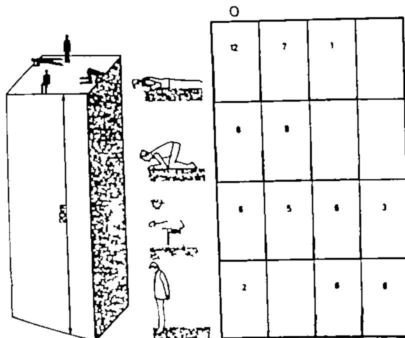


Fig 3 Magnitude of subjective height vertigo as related to body position, being the greatest with upright stance where maintaining postural balance is comparatively most difficult. Scalings (0= none· = moderate ++=medium +++=severe height vertigo) of 20 volunteer subjects not particularly susceptible to height vertigo

2. Height vertigo should be induced either by downward or upward gaze (provided visual conditions are held constant) because it is the distance rather than the visual direction that is critical (psychophysical experiment)

3 Height vertigo should appear at a distance of about 3 m should increase with increasing distance and saturate at a height below 15–20 m where it should be maximal in intensity (psychophysical and posturographic experiments)

4 Nearby stationary contrasts in the periphery of the visual field should improve the height vertigo sway because the retinal periphery dominates postural balance (posturographic experiments)

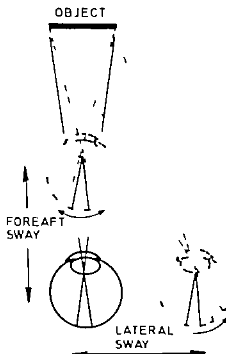
5 Extreme head tilt (by which the otoliths are brought out of their optimal working range) or additional disturbances of the somatosensors (Standing on a soft foam rubber platform) should enhance the height vertigo sway because the 'false' visual signal should be accorded a disproportionately greater influence (posturographic experiments)

The psychophysical experiments were performed under natural conditions on high buildings We were aware of standardization problems which might result by deviating from usual laboratory procedures but felt that field conditions were necessary for proper validation of our hypothesis.

EXPERIMENTS AND RESULTS

Experiment I Influence of body position

Twenty healthy volunteer subjects (10 male 10 female average age 30) who were not particularly susceptible to height vertigo and were unfamiliar with the hypothesis took part in this experiment. They were led separately to the extreme outer edge of the flat roof of a building 20 m high (Fig. 3) In a random order they assumed four different positions, upright stance unsupported sitting positioning on elbow and knees and lying position. They were required to look down for a period of 1 min and to scale the overall distressing effects or height vertigo experienced in each condition Using a simple scaling method they had the choice between. none (−) moderate (+) me



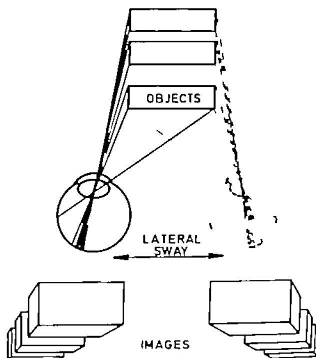


Fig 2 Differential effects of lateral and fore–aft head sway on retinal image shift of a stationary object. Geometrical representation of this shift for two-dimensional and three-dimensional objects (absolute and relative motion parallax respectively)

tural stabilization Experiments with the swaying room demonstrated that optokinetically induced head sway saturates at amplitudes greater than 2 cm which is indicative of proprioceptive interference (Bles et al 1977)

The trigonometrical model developed for afferent motion perception during lateral horizontal body sway must also be valid for efferent motion perception since the thresholds of movement detection are about the same with eye pursuit and stabilized retinal images It must also hold for fore–aft sway in which the retinal images vary in size with the sways (Fig 2) Involvement of eye torsion —in which the retinal shift is independent of the distance between eye and object—can be consequential for posture in case of great lateral (rotational) sway amplitudes only

It seems reasonable to conclude that the displacement angle on the retina, dependent as it is upon distance cannot be a determinant of the net compensatory sway There must be higher order depth constancy mechanisms similar to those for size and motion constancy involved in compensating for the retinal deficiency This is evident in a normal structured surround when the body sways in front of several stationary objects at different distances Here monocular and binocular depths cues as well as motion parallax may be used to stabilize posture (Fig. 2) The gain of the motor reaction to a visually sensed sway requires a precise multisensory calibration based on all mechanisms subserving self motion perception The retinal shift of about 20 min of arc seems to be a minimal requirement for the visual afferent contribution

For the validation of our hypothesis that height vertigo is based on visual destabilization of free stance when the distance between eye and object becomes critically large a number of propositions must be demonstrated with psychophysical (paper I) and posturographic (paper II) experiments

I The occurrence of height vertigo should be clearly related to body position It should be the greatest with the erect stance when maintaining balance is most difficult and minimal in the lying position (psychophysical experiment)

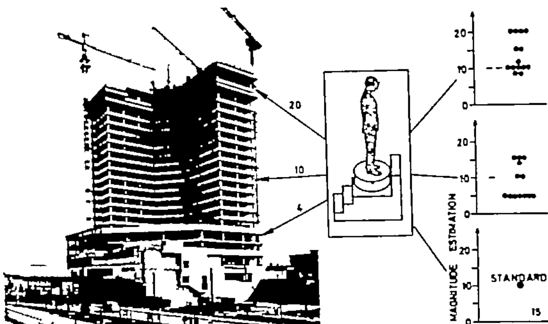


Fig. 5 Saturation of subjective height vertigo at increasing altitudes (20, 50 and 100 metres 4th, 10th, and 20th floor respectively). Magnitude estimations of 15 subjects are not significantly different for the three altitudes. The fourth floor (20 metres) served as the standard situation (modulus with an arbitrary value of 10)

desired perceptual cues provided by windows and visible ceilings were prevented by restriction of peripheral vision to a total field of 120 degrees in diameter Subjects were blindfolded and taken in an outside elevator to the fourth floor at an altitude of approximately 20 m Thereupon via a staircase they stepped onto the stabilometer platform which stood adjacent to the outer wall of the building. The blindfolds were removed and the subjects suddenly exposed to the height vertigo inducing situation during which they were required to look down for a period of one minute A security belt was used as a precautionary measure The fourth floor condition served as the standard situation for further magnitude estimations of subjective vertigo (modulus with an arbitrary value of 10) Posturographic measurements were performed of the fore–aft and lateral body sway with eyes closed and eyes open as well as obtaining the subject's scalings of height vertigo severity All s were then taken from the fourth to the twentieth and finally to the tenth floor representing an altitude of about 100 and 50 m respectively Technical factors within the building site prevented randomization of the order of exposure to the different heights

As depicted in Fig. 5 statistically there was no significant difference among the height vertigo intensities experienced at altitudes of 20 50 or 100 m, although some *S*'s gave greater estimations on the twentieth floor For most subjects vertigo was already maximal on the fourth floor Thus subjective height vertigo seems to be saturated and maximal at a height of about 20 m Habituation may have contaminated the data, however This is suggested by the slight decrease of the mean scaling value at floor 10 compared with the standard at floor 4

The stabilograms of postural sway taken under these natural environmental conditions were affected by occasional wind and an extensive body tremor due to acrophobia (Fig. 6) This tremor had a frequency of 5–10 Hz and did not represent the visually induced sway of height vertigo because it continued

SUBJECTIVE HEIGHT VERTIGO

gaze up

gaze down

Fig 4 Relative influence of downward versus upward gaze on occurrence of subjective height vertigo by use of the open cabin of a fire-brigade ladder in front of a 24 metre tower Scalings (0= none += moderate ++ medium) of 16 volunteer subjects revealed. Height vertigo can be induced with gaze up Distance rather than the direction of gaze appears to be most critical

dium (++) and severe (+++) height vertigo The subjects demonstrated no problems in understanding and performing this task.

As seen in Fig 3 maximal height vertigo occurred with upright stance where most of the subjects reported severe (8) or medium (7) vertigo Only two persons felt free of any distressing effects while standing at the edge Sitting although objectively quite dangerous was tolerated much better Only three had severe and six medium vertigo The elbow–knee position was even less disturbing with no subject having severe and only 4 medium vertigo Finally the lying position was best tolerated and did not evoke height vertigo symptoms in the majority of the subjects Thus the magnitude of subjective height vertigo obviously depends upon body position and is the greatest with upright stance

Experiment II Influence of downward vs upward gaze

Sixteen voluntary subjects (9 male 7 female average age 28) were blindfolded and separately taken to an altitude of 12 m in the small open cabin of a fire brigade ladder in front of a 24 m tower (Fig 4) Upon the experimenter's command the subjects looked at either the upper or lower end of the tower Peripheral vision was restricted to an angle of about 120 degrees by use of a funnel attached to the head This was to exclude possible cues from the cabin s contrasts There were no moving objects such as clouds which might complicate the experiment by inducing postural instability through optokinetic linearvection Eight subjects started with gaze up and 8 with gaze down As in experiment I scaling was none (−) moderate (+) medium (++) or severe (+++) height vertigo *S* s were mainly height vertigo resistant because knowledge of the stimulus conditions served to weed out the fearful candidates This subject selection process and the low altitude probably were responsible for the low levels of the scaling responses There was no significant difference for gaze-down as compared with the gaze-up conditions (Fig 4) Thus subjective height vertigo seems to be independent of gaze direction and dependent only on the distance between subject and visual scene

Experiment III Influence of altitude

Fifteen volunteer subjects (7 male 8 female average age 32) participated in this experiment on a high building under construction Un-

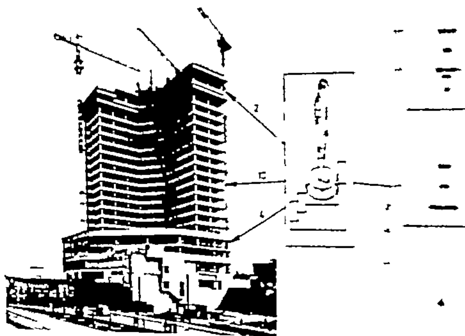


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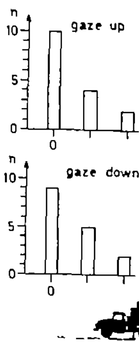
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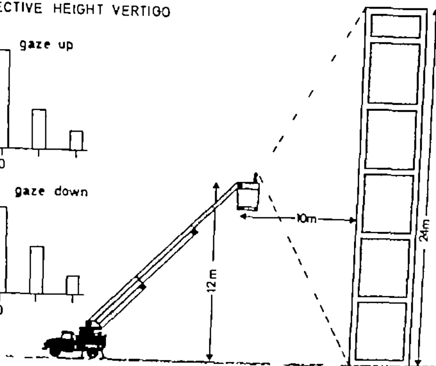


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tigo does not occur on the ground despite the fact that visual targets may be at a great distance because cues from nearby stationary contrasts (provided by peripheral vision) prevent the instigating sensory mismatch

This might become different if the ground is not aligned to the earth horizontal although the relative influence of the angle of incline/decline has not been studied thoroughly In preliminary experiments we found (with great intra- and inter-subject variability) that for a given altitude subjective height vertigo can be induced at an angle of decline of 45–50 degrees and that it progressively increases with increasing slope up to a maximal vertigo With increasing slope peripheral vision provides less information about nearby stationary contours. According to posturographic measurements the development of subjective height vertigo also requires an exposure time of 5 to 30 sec to be saturated.

All visual stimulus characteristics which have an influence on the occurrence and intensity of physiological height vertigo concomitantly affect postural balance Purkinje (1820) was the first to attribute height vertigo symptoms to a postural imbalance of upright station (he stressed the unusual and therefore unadapted stimulation at gaze downward with the stationary visual reference in an imaginary space below foot support) as later was assumed more concretely by Lee & Lishmann (1975) Brandt (1976) Bles et al (1978) and Brandt et al (1978) Purkinje (1820) and Kobrak (1974) erroneously concluded as have modern textbooks that height vertigo mainly represented a disorder in neurasthenic or psychiatric patients

Pogány s (1958) finding, that patients with vestibular dysfunction exhibited a greater susceptibility to height vertigo is now understandable since the erroneous visual signal should have a disproportionately greater sensorial weight when associated with a vestibular lesion This is analogous to our experiments (paper II Bles et al 1979) where induced somatosensory or vestibular systems disturbances (e g. when standing on foam rubber or being carried in a quivering open elevator attached to the outside wall of a building under construction) enhanced subjective height vertigo symptoms

Physical prevention of height vertigo

Knowledge of the stimulus characteristics required for the optimal visual contribution to stabilized postural balance also dictates practical advice related to the reduction of height vertigo in susceptible subjects

1 One should avoid the free upright stance in critical situations at high altitudes. This is done intuitively when grasping for stationary framework or leaning against a wall for support

2. When looking down, one should obtain stationary cues from nearby contrasts in the peripheral visual field (Visual stabilization of posture is served primarily by the retinal periphery while the central retina mainly serves egocentric recognition and pursuit of objects) Staring at moving objects such as clouds increases the danger of falling because additional postural destabilization through linear vection may be induced One should avoid long exposure times as height vertigo usually has a latency taking several seconds to develop Looking through binoculars is very dangerous because it restricts the visual field and introduces the unusual and therefore unadapted magnification factor

3 Body and head position should be adjusted to the gravitational vector because vision will receive a relatively greater sensorial weight (which is undesirable) if the otoliths are displaced beyond their optimal working range by extreme head tilt. It may also be true on the basis of other observations that the feet should be firmly planted on an earth horizontal surface

Physiological height vertigo versus acrophobia

Physiological height vertigo must be differentiated from neurotic acrophobia or height

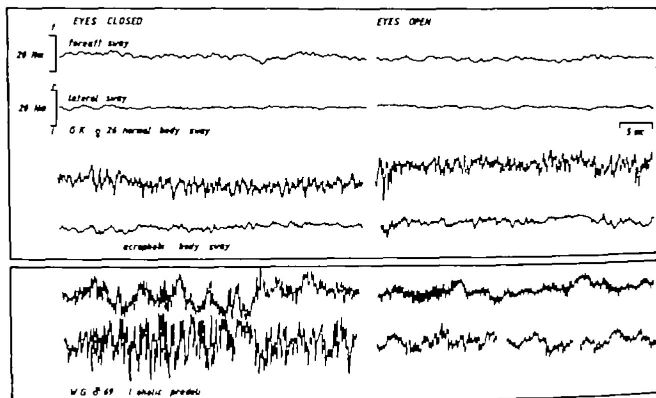


Fig 6 Fore–aft and lateral body sway (stabilogram) of an acrophobic subject on ground as compared with a height vertigo inducing situation on a high building Expectation of exposure to high altitude with eyes closed already causes a postural destabilization and body tremor This tremor whose frequency is 5–10 Hz, is similar to the body tremor of patients suffering from alcoholic predelir but is not related to the low frequency destabilization of physiological height vertigo

with eyes closed Two *S* s became extremely fearful and the experiments had to be interrupted in one subject the security belt was needed to prevent a catastrophic fall

We tested two tight rope artists under the same conditions Although they did not admit to subjective height vertigo they were not willing to perform the experiments without security belts The artists stabilograms exhibited a sway with the same characteristics found in the untrained subjects under identical conditions Paper II will provide posturographic evidence of the body sway amplitudes of normals being dependent on eye–object distance

COMMENT

Height vertigo a distance vertigo

The psychophysical and posturographic data obtained under natural height vertigo conditions were consistent with our hypothesis that height vertigo was due to a visual destabilization of posture when the distance between the eyes and stationary contrasts in the surround becomes critically large The significant increase in body sway amplitudes (paper II Bles et al 1979) reflects a postural imbalance which introduces a real danger of falling from the high position

Our trigonometrical model based on an assumed retinal movement detection threshold of about 20 min of arc predicted that height vertigo would be the most pronounced in the upright stance that it would increase with increasing eye–object distance with a saturation below 15–20 m net altitude and that upward and downward gaze should induce similar responses All of these predictions were confirmed by the scaling experiments Physiological height vertigo appears to be really a distance vertigo because the eye–object distance and not the net altitude or the visual direction is the critical stimulus Distance ver

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Dozent Dr Th Brandt
Neurolog Clinic & clin Neurophy
Krupp II spital
Wittelsdstr 30
4300 Essen
BRD

anxiety in which the altitude acts only as the inducing stimulus situation for a pathological phobic reaction Acrophobia may even become symptomatic on the ground during imagination of high altitudes or if one is exposed to stereographic slides of a downward view from high buildings (Takeya et al 1979) Goethe (1771) was probably the first to report on repeated exposure to height as a valuable therapy of acrophobia which he experienced on top of the Strassburger Münster This later was developed into a behavioral therapy concept of self directed contact desensitization (Ritter 1969 Baker et al 1973) or implosion therapy (flooding) after Marks & Gelder (1967) It is obvious from our experiments that these therapies are concerned with the psychiatric aspects of acrophobia but do not affect physiological height vertigo as described above

ACKNOWLEDGEMENT

We are grateful to Dr R. B Daroff and Dr E Wist for criticism of the manuscript and helpful comments.

This research was supported by Deutsche Forschungsgemeinschaft Br 639/3 Bewegungskrankheit

ZUSAMMENFASSUNG

Der physiologische Hohenschwindel ist eher ein „Distanzschwindel" hervorgerufen durch visuelle Destabilisierung der aufrechten Haltung, wenn der Abstand zwischen Auge und den nachsten stationaren Kontrasten im Gesichtsfeld eine kritische Große erreicht Obwohl der Hohenschwindel allgemein als angstneurotisches Syndrom angesehen wird gibt es eine geometrische Erklarung fur eine physiologische Genese, ein intersensorischer Konflikt der Wahrnehmung von Korperbewegungen Vestibulare und propriozeptive Meldungen widersprechen den visuellen Informationen

Psychophysische Daten bestätigen die Hypothese 1) Hohenschwindel hängt von der Korperhaltung ab und ist beim freien Stand am starksten,) Nicht die absolute Hohe sondern die Auge-Objekt Distanz t entscheidend 3) Mit zunehmender Höhe nimmt der Schwindel zu ist jedoch bei 20 Metern bereits gesattigt

Physiologischer „Distanzschwindel" sollte von der psychopathologischen Acrophobie unterschieden werden Die physiologische Haltungsinstabilitat u ter Höhenschwindelbedingungen kann verhindert werden wenn man darauf achtet, nahe stationare Kontraste im peripheren Gesichtsfeld zu behalten

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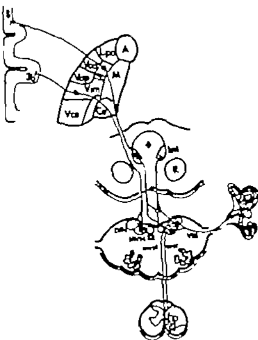


Fig. 1. Diagram showing the main ascending and descending vestibular efferents. The denomination of thalamic nuclei corresponds to Hassler's nomenclature (LVN and MVN lateral and medial vestibular nuclei, mvst and lvst, medial and lateral vestibulospinal tracts)

nith to the different parts of the vestibular nuclear complex. From Lorente de Nó's observations (1938) we know that semicircular ducts project chiefly to the superior and medial nuclei, whereas the utricle supplies the lateral nucleus (or Deiters nucleus) (Fig. 1).

The specific stimulus for the semicircular canals consists in angular acceleration or deceleration of the head which activates the ampullar cristae during movements of the head. The result of this is the nystagmic reaction characterized by slow conjugated deviation of the eyes in the plane of the canal and counter to the direction of movement (slow phase) with a rapid return to the previous position (fast phase). Thus the semicircular canals that Brodal calls kinetic labyrinth records angular movements of the head. This is in agreement with the anatomical data, since the medial vestibular nucleus that receives the impulses from the ampullar cristae projects in turn to the oculomotor nuclei (via the fasciculus longitudinalis medialis) and to the cervical and upper thoracic spinal cord (via the medial vestibulo-spinal tract).

On the other hand the specific stimulus for the hair cells of the utricular macula is the pressure of the otoliths over the particular region where they rest according to the varying attitudes of the head. The utricule—and probably the saccule too—constitute the static or tonic labyrinth. Its impulses are transmitted mainly to the lateral vestibular nucleus of Deiters and thence to the lumbar and sacral spinal cord (via the lateral vestibulo-spinal tract).

The lateral vestibulo-spinal tract terminates entirely according to histological studies of Nyberg-Hansen in the ipsilateral laminae VII and VIII of Rexed of the lumbosacral spinal cord. Laminae VII and VIII contain exclusively the soma of interneuron that of motoneuron being located in lamina IX. However the dendrites of the latter extend to far distant points in laminae VII and VIII (Ramon y Cajal, L. de Nó, Scheibel & Scheibel). There is thus histological data suggesting monosynaptic axodendritic connections between *vestibulo-spinal* fibres and motoneurons as well as axo-somatic contact *with interneurons*. Since the work of Lund & Pompeiano in 1965 various authors have provided *further* physiological evidence in support of this view. From these studies it appears that the descending lateral vestibulo-spinal fibre ends monosynaptically in α-motoneurons (Grillner et al., Shapovalov, Sherrington), γ-motoneurons (Grillner et al., Carli et al., Pompeiano et al.) and interneurons (Grillner et al.). Grillner in 1972 succeeded in provoking monosynaptic EPSPs in ipsilateral α and γ-motoneurons of extensor nucleus of the ankle following stimulation of Deiters nucleus. Only occasionally can monosynaptic EPSPs be recorded in ipsilateral extensor of the knee. Indirect evidence suggests that their effects are exerted almost exclusively on static γ-motoneurons (S. Grillner). Disynaptic

Acta Otolaryngol 89 524–533 1980

THE ROLE OF THE VESTIBULAR SYSTEM IN RELATION TO MUSCLE TONE AND POSTURAL REFLEXES IN MAN

P Molina Negro R. A Bertrand E Martin and Y Gioani

From the Department of Surgery University of Mont eal Canada

(Received November 15 1978)

Abstract The LVST in man seems to provoke through its activation from the utricule in response to displace ment of the center of gravity an increase of the excitabil ity of γ-static motoneurons of extensor muscles of trunk and legs and in a lesser degree of γ-dynamic and α-moto-neurons This contributes to the adjustment of muscle tone for the maintenance of the static standing posture and for the alternative change from the extensor to the flexor phase during locomotion Temporary vestibular hyperfunction provoked by central or peripheral stimula-tion is manifested clinically by vertigo There is during this state a hyperexcitability of γ-static motoneurons with lesser or no change of γ-dynamic and α-motoneurons Suppression of vestibular function by destruction of the labyrinth section of the vestibular nerve interruption of the central vestibular pathways or of the LVST result in disequilibrium with loss of postural control A marked depression of γ-static innervation can be detected in this case The presence of adequate γ-static innervation in one side seems to be contradictory to complete suppression of labyrinthine function in this same side On the other hand absence of response to caloric stimulation in one or both labyrinths does not exclude the possibil ty of ves tibular hyperfunction The study of phasic stretch reflexes appears to be a valid method of measurement of the func tional state of the static labyrinth and of the LVST

The role of the labyrinth and of the vestibular nucleus in postural reactions was demon strated by Sherrington in his remarkable ex periments upon decerebrate animals Transec tion of the brain stem at an intercollicular level provokes in the animal a permanent postural change whose description by Sherrington (1898) is still classic the animal then in ab sence of external stimulation remains with ex tended limbs and neck whether set on its side or prone or supine If placed upright upon its feet it retains the position so given it The weight of the various parts is then supported by the continuous contractions of muscles counteracting gravity Sherrington demon strated that upright stance is maintained by the permanent contraction of antigravity muscles which he called *stretch reflex*

Later he distinguished this *tonic stretch reflex* from the brief contraction of extensor muscles to sudden stretch which he named *phasic stretch reflex* nowadays called *tendon jerks* Sherrington further demonstrated that labyrinthine influences maintain the decere brate rigidity following sectioning of the ves tibular nerve destruction of the nucleus of Deiters or sectioning of the lateral vestibulo spinal tract (LVST) suppress decerebrate rigidity In 1906 Sherrington wrote each labyrinth maintains tonus especially in the neck and trunk muscles and in the extensor abductor limb muscles of the homonymous side"

Since both the tonic and the phasic reactions are a manifestation of the same myotatic or stretch reflex its modification in health or in disease is always parallel Hence analysis of tendon jerks much more easily reproducible and measurable than the tonic stretch reflex provides an index of the functional state of postural reactions Alteration of labyrinthine function will induce changes in postural reac tions that can be detected and measured by analysis of tendon jerks

Anatomical and Physiological Studies on Vestibulo-spinal Projections

There is a precise topographical arrangement of the projections from each part of the laby

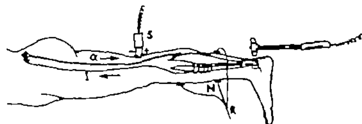


Fig. 3 The position of the recording and stimulating electrodes as well as the pathways involved in the myotatic reflex are represented

the ankle joint is passively relaxed this minimum degree of γ static innervation due to the force of gravity will further diminish and the response thus obtained (which we call AI Fig. 4) will be proportionately reduced In a normal individual the ratio AI/AR indicates the proportion of γ-static versus γ-dynamic innervation whereas the index AI/M will indicate the proportion of γ-static versus the total pool of motoneurons An increase or decrease in LVST activity will respectively increase or decrease the value of AI/AR and AI/M

The Jendrassik manoeuvre has been shown to provoke an increase in facilitatory supra segmentary influences primarily of γ-dynamic and static motoneurons (J Paillard) AI and AR are effectively increased during the manoeuvre in normal subjects The degree of augmentation is inversely proportional to the degree of excitability of motoneurons Thus in spastic patients in which the loop is already saturated the effectiveness of the Jendrassik manoeuvre to increase AI—and still more the AR—is reduced or even abolished Inversely desaturation of the γ loop by a lack of activity of facilitatory influences will relatively increase the effectiveness.

The excitability of α-motoneurons is measured by the index H/M and by the recovery curve of the H reflex (J W Magladder) Passive relaxation of the ankle joint or the Jendrassik manoeuvre induces very little if any modification of the value of H and did not modify the duration of the refractory periods of motoneurons at all (N Poirier et al) Increase in α-motoneuron excitability is manifested by an increase in H/M and a shortened refractory period Conversely a reduction in the α-motoneuron excitability induces a fall in H/M and a lengthening of the refractory period

MATERIAL

In order to determine the function of the LVST in man we compared the values of stretch reflexes in 54 healthy persons with those of patients with hyper and hypofunction of the vestibular system The former group

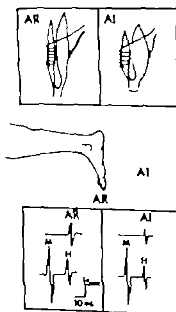


Fig. 4 With the gastrocnemius in completely relaxed position the mechanically induced reflex AI is smaller than the reflex obtained with the foot in natural resting position called AR. The H reflex and the M response have the same value in both instances.

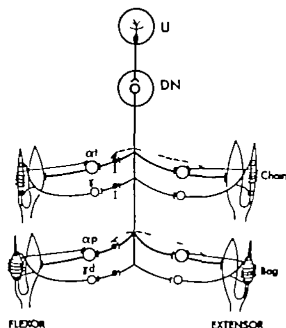


Fig 2 The main connections of the LVST with spinal motoneurons are presented (*u* utriculum *DN* Deiters nucleus *αt* alpha tonic motoneurons *αp* alpha phasic motoneurons *γs* gamma static motoneurons *γd* gamma dynamic motoneurons)

EPSP can also be provoked in α motoneurons of ipsilateral extensor of the ankle and in a group of flexor of the toe Disynaptic IPSPs can also be evoked in ipsilateral α and γ-motoneurons of knee and ankle flexors as well as in a small group of hip extensors

Disynaptic EPSPs can be evoked in contra lateral hip knee and ankle extensors through ipsilateral interneurons Finally trisynaptic IPSP can be evoked in contralateral flexor motoneurons (Grillner & Hongo)

In relation to the effect on the spinal reflex pathways Grillner remarks that the vestibulo-spinal tract facilitated generally reflex areas which give the same effect to α motoneurons as it gives itselfs i e *excitation of extensors and inhibition of flexors* The effects are exerted bilaterally

Fig 2 summarizes the previous experiences

From these experiences it appears—according to Gernandt (1967)—that the fact that two antagonistic *spinal* reflex responses the extensor gastrocnemius and the flexor tibialis anticus are facilitated suggests that vestibular (LVST) influences at the spinal segmental level contribute to co-contractional muscular patterns necessary to the pillar-like stability of the weight-bearing limb (B Gernandt) On the other hand Grillner & Hongo (1972) concluded that the function of the (lateral) vestibulo-spinal tract during locomotion is to coordinate and adjust the extensor activity to an optimal level during each cycle presumably through the influence of cerebellum or its nuclei (Grillner & Hongo)

METHOD

The stretch reflex has a phasic and a tonic component The phasic reflex can be elicited mechanically by a sudden tap over the tendon This is the case of tendon jerks (Fig 3) The stretching of a muscle spindle monosynaptically excites the α-motoneurons innervating the same muscle which will immediately contract This reflex (A) and its modification by various manoeuvres can be easily recorded and measured by applying the technique described by Paillard On the other hand due to the fact that the threshold of Ia fibres is lower than that of the motor axons it is possible to provoke a phasic stretch reflex by electrical stimulation of the former beneath the muscle spindles The reflex thus obtained is called (according to Hoffman) the H reflex (Fig 3)

The amplitude of A depends mainly on the state of excitability (readiness to fire) of the muscle spindles conditioned in turn by excitatory and inhibitory suprasegmentary impulses The more important of the former is precisely the LVST Its action induces an increase in tension of nuclear chain intrafusal fibres through activation of static γ motoneurons On the other hand due to the α-γ linkage the amplitude of A will also be in relation with the degree of excitability of α motoneurons The tendon jerk obtained in a resting position will be called AR (Fig 4) This value depends on the degree of excitability of α-phasic and α-dynamic motoneurons at rest added to the degree of excitability of γ-static motoneurons in a natural resting position If

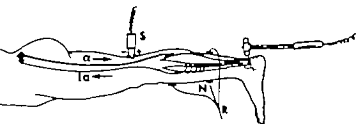


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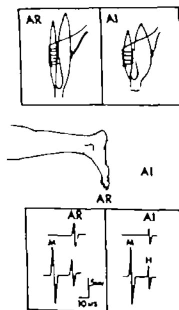


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Table I *Modification of contralateral of stretch reflexes following stimulation and lesion of central vestibular pathways (2 cases)*

	H reflex	H/M index	AI	AR	AI/AR	AI+J	AR+J	AI/H	AI/M	[illegible]	[illegible]
At rest	7	36% M 19 mlvs	3	5	60%	5 (+66%)	8 (+60%)	48%	15%	79%	3
During stimulation	11 (+56%)	57% (+ 1%)	6 (+100%)	10 (+100%)	60%	8 (+33%)	1 (+70%)	54% (+1 %)	31 (+16	[illegible]	[illegible]
After lesion	7 (=)	36% (=)	0.5 (−84%)	2 (−56%)	24% (−38%)	1 (+100%)	4 (+81%)	7% (−35%)	2% (13	31% (−40	[illegible]

comprised 2 patients where electrical stimulation of the central vestibular pathways was performed and one patient who underwent caloric stimulation The later group was integrated with 18 patients treated surgically by vestibular neurectomy 9 cases of antero lateral cordotomy and 2 patients with surgically induced lesions of central vestibular pathways In all the patients selected we have well documented pre per and postoperative recordings of myotatic reflexes

Finally once the electrophysiological criteria of hyper or hypofunction of the vestibular system were established the study of stretch reflexes was extended to an unselected group of patients suffering from various types of vestibular dysfunction

RESULTS

Group I Temporary vestibular hyperfunction during electrical stimulation of central vestibular pathways

During stereotactic procedures at the level of the thalamus and basal ganglia for the control of pain abnormal movements and epilepsy the stimulating electrode is occasionally placed at the level of the tegmental dorso lateral bundle known in the classical literature by the denomination secondary dorsal trigeminal tract of Wallemberg The vestibular nature of this pathway was demonstrated by Hess who described ipsiversive torsion of the feline head during its stimulation Hassler further demonstrated that this bundle [illegible] nates in the neurons of the ventral part [illegible] Deiters nucleus and ends in the [illegible] ventro-intermedius (Vim of the [illegible] Low intensity and high frequency [illegible] at this level provokes rotatory [illegible] various other postural hallucinations [illegible] study of stretch reflexes during the [illegible] (Table I) shows a marked temporary [illegible] in excitability of α-static and γ-dynamic [illegible] neurons and a moderate increase of [illegible] neurons excitability This effect is [illegible] more pronounced in the contralateral [illegible]

Interruption of this pathway by [illegible] with the fine blunt wire employed [illegible] tactic surgery is followed by co[illegible] hypotonia and loss of postural co[illegible] leg A marked diminution of γ-static [illegible] tion can be demonstrated by the p[illegible] depression of AI and AI/M A m[illegible] duction of γ-dynamic innervation [illegible] fested by a less significant reduc[illegible] value of AR (Table I) Since the v[illegible] H reflex and of the H/M index [illegible] changed the excitability of α-[illegible] seems to be less influenced by t[illegible] the central vestibular pathways [illegible] test became positive after surg[illegible] mained so for months afterwards [illegible] tion in stretch reflexes follows a [illegible] lel with that of the muscle tone [illegible] years later when adaptation is [illegible] the clinical point of view dep[illegible] static innervation is still detectab[illegible]

Table II *Modification of ipsilateral stretch reflexes following caloric (0°) stimulation (1 case)*

	H reflex	H/M	AI	AR	AI/AR	AI+J	AR+J	AI/H	AI/M	AR/H	AR/M
:est	5	31% M. 16 mlvs		5	40%	4 (+100%)	8 (+60%)	40%	12%	100%	31%
ring imulation	6	37%	4	8	50%	5 (+25%)	8 (0)	66%	25%	133%	90%
ference	20%	+6%	100%	+60%	10%	75%	−100%	+26%	+13%	+33%	+25%

roup 2 Temporary vestibular hyperfunction ring caloric (0°) stimulation

his experiment was performed in a single se of benign positional vertigo With the atient lying on his back, the stretch reflexes ere provoked with mechanical and electrical imulation. A few seconds after commencing rigation a marked increase in AI and a oderate increase in AR were detected (Table I). The effect was bilateral but more pro- ounced ipsilaterally Since the values of AI d AR are increased the effectiveness of the endrassik s manoeuvre is proportionally re uced Caloric activation of the labyrinth ould therefore seem to enhance the excitabil y of γ-static and to a lesser degree, γ-dy amic motoneurons

roup 3 Modification of stretch reflexes llowing surgical lesion of the estibular nerve

ighteen patients with various vestibular pa hologies were selected in whom pre and ost-operative studies of myotatic reflexes ere performed The results (Table III) cor espond exclusively to ipsilateral reflexes ob- ained within 2 weeks after surgery From the inical standpoint all patients presented a marked abnormal Romberg test that persisted lthough progressively attenuated for 12 to 18 months But even after years with the pa- ent s feet placed in front of one another and he eyes closed, the Romberg test is still ab- normal when the foot on the side of the neu- ectomy is behind the other

Immediately after surgery there is a marked fall in the value of AI and a much more moder ate decrease in AR and H (Table III) Since Jendrassik s manoeuvre is still strongly posi tive for AI after surgery it appears that the suppression of vestibular function provokes a selective diminution of the excitability of γ-static motoneurons Repeated studies after periods of up to 5 years following surgery

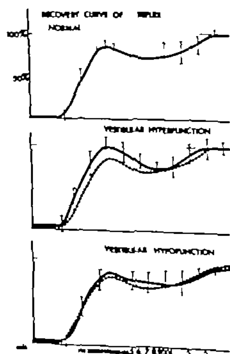


Fig 5 Recovery curve of the H reflex in normal persons and in patients with hyper and hypofunction of the vestibular system The dotted lines in the second and third curve correspond to the average normal result.

Table I *Modification of contralateral of stretch reflexes following stimulation and lesion of central vestibular pathways* (2 cases)

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Interruption of this pathway by opening with the fine blunt wire employed in stereotactic surgery is followed by contralateral hypotonia and loss of postural control in the leg A marked diminution of γ-static innervation can be demonstrated by the pronounced depression of AI and AI/M A moderate reduction of γ-dynamic innervation is manifested by a less significant reduction in the value of AR (Table I) Since the values of the H reflex and of the H/M index remain unchanged the excitability of α motoneurons seems to be less influenced by the lesion of the central vestibular pathways Romberg's test became positive after surgery and remained so for months afterwards The alteration in stretch reflexes follows a course parallel with that of the muscle tone But even 10 years later when adaptation is perfect from the clinical point of view depression of γ-static innervation is still detectable

Table II Modification of ipsilateral stretch reflexes following caloric (0°) stimulation (1 case)

	H reflex	H/M	AI	AR	AI/AR	AI+J	AR+J	AI/H	AI/M	AR/H	AR/M
At rest	5	31% M, 16 mV		5	40%	4 (+100%)	8 (+60%)	40%	12%	100%	31%
During stimulation	6	37%	4	8	50%	5 (+25%)	8 (0)	66%	25%	133%	50%
Increase	20%	6%	+100%	+60%	+19%	−75%	−100%	+26%	+13%	+33%	+25%

roup 2 Temporary vestibular hyperfunction ring caloric (0°) stimulation

his experiment was performed in a single se of benign positional vertigo With the tient lying on his back, the stretch reflexes ere provoked with mechanical and electrical mulation A few seconds after commencing igation a marked increase in AI and a oderate increase in AR were detected (Table I). The effect was bilateral but more pronounced ipsilaterally Since the values of AI d AR are increased the effectiveness of the endrassik s manoeuvre is proportionally reiced. Caloric activation of the labyrinth ould therefore seem to enhance the excitabil-y of γ-static and to a lesser degree γ-dy amic motoneurons

roup 3 Modification of stretch reflexes ollowing surgical lesion of the estibular nerve

ighteen patients with various vestibular pa hologies were selected in whom pre and ost-operative studies of myotatic reflexes were performed The results (Table III) cor espond exclusively to ipsilateral reflexes ob- ained within 2 weeks after surgery From the linical standpoint all patients presented a marked abnormal Romberg test that persisted lthough progressively attenuated for 1 to 18 nonths. But even after 2 years with the pa ent s feet placed in front of one another and he eyes closed the Romberg test is still ab-normal when the foot on the side of the neu-rectomy is behind the other

Immediately after surgery there is a marked fall in the value of AI and a much more moderate decrease in AR and H (Table III) Since Jendrassik s manoeuvre is still strongly positive for AI after surgery it appears that the suppression of vestibular function provokes a selective diminution of the excitability of γ-static motoneurons Repeated studies after periods of up to 5 years following surgery

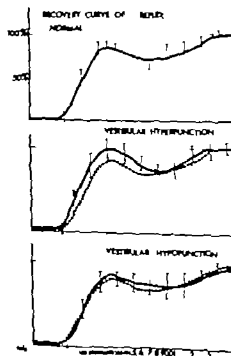


Fig 5 Recovery curve of the H reflex in normal perso and in patients with hyper and hypofunction of the ve tibular system The dotted lines in the second and th curve correspond to the average normal result.

Table III *Modification of ipsilateral stretch reflexes following vestibular neurectomy (18 cases)*

	H	H/M	AI	AR	AI/AR	AI+J	AR+J	AI/H	AI/M	AR/H	AR/M
Before	8.3	48% M 17 1 mlvs	4	6	66%	6 4 (+60%)	7 (+16%)	48%	23%	7?%	35%
After	6 8	39%	2 1	5.5	38%	4 2 (+100%)	6 (+9%)	30%	12%	80%	3?%
Difference	−18%	−9%	−48%	−9%	−?8%	−40%	−7%	−18%	−11%	+1?%	−3%

show a progressive attenuation of this effect It is our opinion that if the myotatic reflexes are normal before surgery and the surgical suppression both of the vestibular nerve and of the ganglion of Scarpa are complete the decrease in γ static innervation will remain definitive Should a complete normalization of reflexes occur after surgery the possibility of an incomplete ablation must be considered This possibility is still greater if there is recurrence of vertigo

Group 4 Modification of stretch reflexes after antero-lateral cordotomy

The LVST is situated immediately in front and slightly medial to the lateral spino-thalamic tract Sectioning of the latter for relief of pain is therefore accompanied by interruption of the former Hypotonia and loss of postural control in the ipsilateral leg always occur following antero-lateral cordotomy The paresis that occasionally occurs when a too posterior lesion encroaches on the lateral cortico spinal tract is also accompanied by immediate hypotonia and imbalance but the characteristic hyperreflexia and spasticity of the pyramidal syndrome will appear shortly after

The study of stretch reflexes following antero-lateral cordotomy shows the most dramatic and selective loss of γ-static innervation (Table IV) Indeed for a period of about ? to 4 months the ankle jerk in the maximal relaxation position (AI) remains absent whereas the diminution of the ankle jerk in a resting position (AR) is minimal The excitability of α-motoneurons is not affected significantly by transection of the LVST

DISCUSSION

From the results presented it appears that vestibular hyperfunction provoked by caloric stimulation of the labyrinth or by electric stimulation of central vestibular pathways is accompanied clinically by various postural hallucinations and primarily by vertigo The study of stretch reflexes in these states demonstrates the hyperexcitability of extensor

Table IV *Modification of ipsilateral stretch reflexes following anterolateral cordotomy (9 cases)*

	H reflex	H/M	AI	AR	AI/AR	AI+J	AR+J	AI/H	AI/M	AR/H	AR/M
Before	6.5 ml M 19 mlvs	34%	2.5	4.5	55%	4 (+60%)	7 (+55%)	13%	38%	?3%	69%
After	6	31%	0	3	0%	0	4 (+?5%)	0	0	15%	50%
Difference	0	0	−100%	−33%	−100%	0	−43%	−100%	−100%	35%	−28%

Table V *Comparison between stretch reflexes in normal (54) vestibular hyperfunction (13) and vestibular hypofunction (9)*

	H	H/M	AI	AR	AI/AR	AI+J	AR+J	AI/H	AI/M	AR/H	AR/M
Normal	6.5	34%	3.5	5	70%	5.7 (+62%)	6.2 (+24%)	53%	18%	76%	26%
Hyper	8.6	54.6	5	6.2	80%	6.5 (+30%)	7.6 (+22%)	58%	31%	72%	39%
Hypo	6.6	47.3	1.5	3.3	45%	2.8 (+86%)	4.9 (+48%)	22%	10%	50%	23%

lumbo-sacral motoneurons strongly predominant for γ-static motoneurons and less pronounced for γ-dynamic and α-motoneurons (Fig. 6).

On the other hand vestibular hypofunction following interruption of central vestibular pathways vestibular neurectomy or sectioning of the LVST at the spinal level becomes clinically manifest by the loss of postural control on the lesion side. Careful analysis in cases of minimal involvement shows that the sluggishness of the leg is due to hypotonia of extensor antigravitatory muscles accompanied *by the loss of postural reflexes* i.e. of the tonic component of the stretch reflex. This constitutes what we call an abnormal Romberg test of negative type. Study of the phasic *stretch reflexes shows a dramatic fall in excitability* of γ-static motoneurons that can persist for months or even years. A less pronounced reversible fall in excitability of γ-dynamic and α-motoneurons is also noticed (Fig. 7). Furthermore in cases of vestibular neurectomy when the fall in the value of AI is moderate or absent, the possibility of an incomplete *neurectomy must be considered*.

We are now performing a systematic study of stretch reflexes in every case of vestibular dysfunction where diagnosis is not possible and in every case in which surgery of the laby

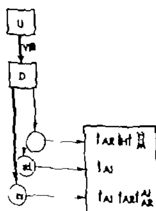


Fig. 6. In cases of vestibular hyperfunction the impulses from the utriculum (*U*) and the lateral *vestibular nucleus* of Deiters (*D*) provoke state of hyperexcitability of alpha motoneurons (γd), gamma dynamic (γd) and gamma static (γs) motoneurons. The values of AI, AR and H are at these circumstances increased in relation to nor

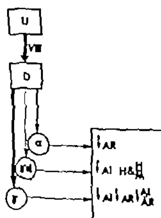


Fig. 7. In cases of vestibular hypofunction the excitability of α and gamma motoneurons is decreased. The values of AI AR and H are the same cases reduced in *relation to normal*.

rinth or vestibular nerve is considered Table V shows the preliminary result of this study series In 13 cases in which the dominant symptom is vertigo the pattern of stretch reflexes corresponds to that of vestibular hyperfunction In 9 cases of vestibular dysfunction in which chronic disequilibrium with unilateral positive Romberg test of deficitatory type is present the pattern of myotatic reflexes corresponds to vestibular hypofunction On the other hand there is no correlation between the electronystagmography and the stretch reflexes Indeed a hyporeflexive labyrinth can be found in cases of vertigo and/or disequilibrium in which the pattern of reflexes is of hyperfunctional type

A certain tendency towards a pattern of hyperexcitability of α-motoneurons can be found in the hyperfunctional type according to the recovery curve of the H reflex (Fig 5) whereas a strictly normal curve is found in the hypofunctional type

ZUSAMMENFASSUNG

Der Fasciculus vestibulo-spinalis lateralis (FVSL) scheint beim Menschen durch seine Erregung aus dem Utriculus in Antwort auf Verlagerungen des Gravitatszentrums eine Erhöhung des Erregungsniveaus der statischen γ-Motoneurone der Streckmuskulatur des Rumpfes und der unteren Extremitaten sowie wenn auch weniger ausgeprägt der dynamischen γ und der α-Motoneurone zu erzeugen Dies tragt dazu bei den Muskeltonus zur Beibehaltung der statischen Aufrechtposition und zum alternierenden Wechsel zwischen der gestreckten und gebeugten Phase wahrend der Fortbewegung zu regulieren

Eine orubergehende estibulare Uberfunktion bedingt durch zentrale oder periphere Stimulation außert sich klinisch durch Schw ndel Wahrend dieses Zustandes beobachtet man eine Ubererregbarkeit der tatischen γ-Motoneurone während die dynamischen γ und die α-Motoneuronen wenig oder gar nicht verandert sind

Eine Aufhebung der vestibularen Funktion durch Zerstörung des Labyrinths Durchtrennung des Nervus vestibularis, Unterbrechung der zentralen vestibularen Verbindungen oder des FVSL hat eine Gleichgewichtsstörung mit Verlust der Lagerungskontrolle zur Folge In diesem Fall wird eine erhebliche Abnahme der tatischen γ-Innervation registriert

Das Vorhandensein einer adäquaten statischen γ-Innervation auf einer Seite scheint mit einer ollstandigen Aufhebung der Labyrinthfunktion auf der gleichen Seite im Widerspruch zu stehen Andererseits schließt das Fehlen einer Antwort auf kalorischem Reiz in einem oder beiden Labyrinthen die Möglichkeit einer vestibulären Uberfunktion nicht aus

Die Beobachtung der phasischen Muskeldehnungsreflexe scheint eine angemessene Methode zur Beurteilung des Funktionszustandes des statischen Labyrinths und des FVSL zu sein

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P Molina-Negro M.D Ph.D
1560 Est Rue Sherbrooke
Montreal P Q
Canada H2L 4M1

Acta Otolaryngol 89 534–540 1980

THE MECHANISM OF PHYSIOLOGICAL HEIGHT VERTIGO

II *Posturography*

Willem Bles Theo S Kapteyn Thomas Brandt[1] and Friedrich Arnold[1]

From the E.N.T Department Free University Hospital Amsterdam The Netherlands and the Neurological Clinic with Clinical Neurophysiology Krupp Hospital Essen Federal Republic of Germany

(Received April 23 1979)

Abstract In order to validate the hypothesis that height vertigo is based on visual destabilization of free stance when the distance between eye and object becomes critically large several of its consequences were demonstrated in posturographic experiments. (1) Visual signals conflicting with simultaneous vestibular and somatosensory inputs provided by sinusoidally tilting rooms may destabilize postural sway in the fore–aft as well as in the lateral direction. (2) In natural surroundings sway amplitudes increase with increasing eye–object distance up to 5 meters Thus teleologically subjective height vertigo serves as an appropriate warning signal to withdraw the body from a stimulus situation inducing postural imbalance. (3) Postural height vertigo problems can be alleviated (*a*) by adjusting the head relative to the gravitational vector and (*b*) by the presence of nearby stationary contrasts in the visual periphery according to the dominance of retinal periphery for dynamic spatial orientation.

The hypothesis in paper I Theoretical Approach and Psychophysics (Brandt et al 1979) provided the provocative prediction that at free upright stance the body sway will increase with increasing distance between the eyes and the nearest stationary objects within the visual scene The present paper provides posturographic evidence supporting this hypothesis

It has been argued that physiological height vertigo might be due to a specific insufficiency of the visual system the limited resolution Δ of the retina Head movements cause a retinal slip of stationary visual targets The retinal shift α of a viewed stationary object in the environment depends on the lateral head displacement Y and on the eye–object distance X A head or eye displacement Y is not perceived for $\alpha < \Delta$ In this latter situation there is no visual cue that could be used for postural compensation of this movement The lack of a visual cue should result in larger disinhibited sway amplitudes at greater eye–object distances

A series of experiments was designed to test whether height vertigo is associated with a visual destabilization of postural balance

METHODS AND RESULTS

Stabilometry

The fore–aft and lateral body sway were separately measured by use of a stabilometer (Kapteyn & de Wit 1972) at free upright stance Stabilograms were both recorded by a strip chart recorder (Siemens EM 81 80) and stored on a magnetic tape recorder (Philips Analog-7) for further data analysis Apart from the high frequency components the stabilograms very closely represent the movements of the centre of gravity and of the head as verified by means of a TV detection system when compared with the simultaneous stabilogram (Bles & de Wit 1976 Kapteyn 1973)

In order to enhance disproportionally the particular sensory weight of the visual information for postural control some of the experiments were performed with one or two pieces of foamr[illegible]

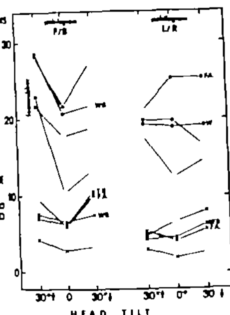


Fig. 1. The Root Mean Square (RMS) of lateral and fore-aft stabilograms obtained with upward or downward inclination of the head 30° out of the normal position (normal head position: ~15° tilted forward). The 4 subjects were standing with eyes closed both without ( ) and with (O) foamrubber on top of the stabilometer.

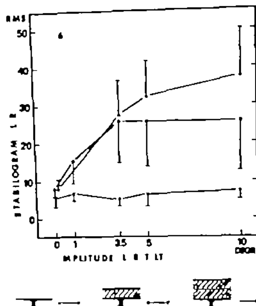


Fig. 2. RMS values (arbitrary units ±1σ) of the lateral stabilogram obtained under sinusoidal lateral movement of Tilting Room I (freq. 1/45 Hz, ampl. 1, 3.5, 5 and 10°). The 6 subjects were standing both without (×), with single (O) and with double (Δ) foamrubber on top of the stabilometer.

40 g/dm³) placed on top of the stabilometer covered by a rigid plate. This makes the exteroceptive information of the ankle joints even less effective in maintaining postural balance.

Tilting rooms

In the laboratory the effects of misleading moving visual signals on posture were investigated by use of stabilometry during artificial movements of the viewed environment.

Tilting Room I is a motor-driven device (2.5×1.5×2 m) tilting laterally around an axis at ankle height with the subject standing on the stabilometer in front, the entire visual field covered by the stimulus device (Bles & de Wit, 1976). The room was tilted sinusoidally at a frequency of 1/45 Hz and amplitudes of either 1, 3.5, 5 or 10°.

Tilting Room II (Tönnies, Freiburg i.Br.) is completely closed except for a hole in the centre of the floor where the stabilometer is placed. This allows for lateral as well as fore-aft optokinetic stimulation (Bles, 1979). Stimulus frequency was 1/40 Hz with amplitudes of either 2, 4, 8 or 12°.

The subjects were requested to stand upright keeping optimal balance for at least three stimulation periods.

Computer-analysis of the stabilograms

The analysis of the stabilograms was performed using a PDP-8I computer system.

Fourier analysis of postural sway is very common nowadays (Dichgans et al., 1976, Leroux et al., 1973). The stationarity of the stabilogram which is required, however, is unlikely to be achieved during a one-minute registration period. Therefore we preferred a computerized analysis of sway amplitudes using Root Mean Squares (RMS) as a measure of stability (Kapteyn & Bles, 1976).

According to the hypothesis developed in paper I of this study, we would expect to find a low frequency high amplitude body sway in

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In order to enhance disproportionally the particular sensory weight of the visual information for postural control some of the experiments were performed with one or two pieces of foamrubber (height 10 cm spec wt

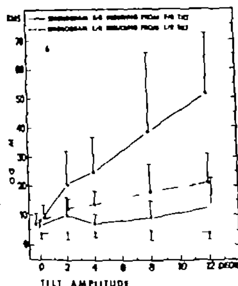


Fig. 4. Mean RMS values (arbitrary units, $\pm 1\sigma$) of lateral and fore–aft stabilograms. Both the lateral and the fore–aft stabilograms were obtained from lateral resp. fore–aft sinusoidal tilt of Tilting Room II (freq. 1/40 Hz, ampl. 2, 4, 8 and 12°). The 6 subjects were standing both without ( ) and with (O) foamrubber on top of the stabilometer.

attached to the head and restricting the visual field to 75°. For each condition six stabilograms were recorded, no, one or two layers of foamrubber with or without a funnel. It must be emphasized that under these natural conditions it was not possible to control all stimulus parameters of the visual scene or to avoid occasional presence of a slight breeze.

As can be seen in Fig. 3 the instability is already maximal at an eye–object distance of 25 m. No significant difference between the RMS values for the different test conditions obtained at 25, 75 and 200 m could be established. This is also the case when peripheral vision is present. However, when using foamrubber, the levels of instability (RMS values) obtained with peripheral vision are below the levels found with the restricted visual field ($p < 0.01$).

The RMS of the stabilograms obtained in the 'eyes-closed' conditions is of the same order of magnitude as the RMS obtained with eye–object distances of at least 25 m without peripheral vision. Ranking the subjects according to the RMS in both the balcony and tilting room experiments gives a coefficient of rank correlation of 0.82, indicating that the role of vision in postural stabilization is subject-dependent. Since the postural instability is already maximal at a distance of 25 m the data of experiment I do not sufficiently determine the saturating relationship between eye–object distance and sway amplitudes below 25 m. Therefore a second experiment was designed for testing the influence of small distances as well as the differential effects of optokinetic stimulation on fore–aft and lateral body sway.

Experiment II. Lateral and fore–aft body sway at various distances (0.5 to 25 m) and proprioceptive interference

Six healthy subjects (4 male, 2 female, age 20–4?) participated in this experiment.

First they were tested in Tilting Room II both with and without foamrubber. The results are shown in Fig. 4. There is a remarkable discrepancy with the results of Expt I. Although the RMS obtained with one layer of foamrubber in Tilting Room II is less than that found in Expt I, the subjects could not perform the test on double foamrubber. Most of the subjects preferred to have some assistance available even with only one layer of foamrubber because they felt unstable and sometimes experienced vertigo.

It is interesting to note that the subjects were much more sensitive to the fore–aft than to the lateral tilt.

The Tilting Room II experiment was followed by a more natural stimulus situation for testing the influence of the distance below 25 m under static conditions. In this experiment a university lecture hall was used to provide different eye–object distances, thus eliminating the possibility of wind influencing the results. By a suitable choice of illumination and the use of an adapted funnel the eye–object distance could be varied up to 25 m. The distances in the lecture hall at which stabilograms were recorded were 0.5, ...5, 5

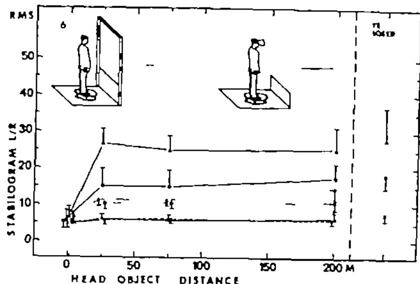


Fig 3 Mean RMS values (arbitrary units, $\pm 1\sigma$) of lateral stabilograms obtained at different eye–object distances (0.5 25 75 and 200 m) without (—) and with ( ) the presence of nearby stationary contours in the periphery of the visual field. The eyes closed condition was tested as well. The 6 subjects were tested without (×), with one (O) and with two (Δ) layers of foamrubber on top of the stabilometer

conditions with considerable eye–object distance This makes the choice of the RMS as a measure of stability in the following experiments acceptable Since the RMS values represent an average over a certain period they cannot be used for calculation of the net head movements (Y) But an increase in RMS values of the body sway is associated with an increase in head movements The RMS values were computed from the last 45 sec of the one minute recordings in experiment I and from the last 40 sec in experiment II In the tilting room experiments the RMS was computed for the second stimulus sinusoid in order to avoid possible transients

Pilot Experiment Effects of head position on postural stabilization

Frequently the head is bent or extended in height vertigo inducing stimulus conditions when looking up or down high structures The net influence of the head position relative to the gravitational vector on posture was measured with eyes closed because with eyes open effects will be contaminated by additional changes in visual cues

As can be seen in Fig 1 upward or downward inclination of the head by approx 30° out of the normal position affects body sway—at least in the fore–aft direction Stabilization is best with the otoliths in their normal working position (normal head position tilted forward ~15°)

Experiment I Lateral body sway at various eye–object distances (0.5 to 200 m) influence of peripheral vision and proprioceptive interference

Six healthy students (4 male 2 female age 21–24) participated in this experiment They first were tested in Tilting Room I while standing both with and without foamrubber to check on the proprioceptive interference during visual destabilization

The results in Fig 2 show that left/right instability of the stabilogram without foamrubber does not increase very much despite the increasing amplitude of the room tilt With foamrubber a maximum level of RMS values indicating proprioceptive interference is reached already at an amplitude of 3.5° of room tilt With double foamrubber this occurs at a higher level as was to be expected The considerable standard deviations suggest large interindividual differences

Subsequently by placing the stabilometer on balconies of a high building it was possible to vary the distance between the eye and the visible surroundings from 0.5 25 75 up to 200 m Nearby stationary visual cues from the retinal periphery (balustrade and ceilings of the balcony) could be occluded by a funnel

ye closure during pathological vestibular vertigo. This explanation in fact fits earlier results in patients with postconcussional dizziness or peripheral lesions when exposed to artificial surround motion in Tilting Room I (Bles 1977 Bles et al 1977) These patients probably because of the lesional dysfunction of the vestibular system exhibited similar symptoms even without use of foamrubber

The lower saturation levels of RMS values (see Expt I) as obtained in the peripheral vision-foamrubber conditions are visually determined and therefore can be interpreted as levels of visual interference This is true because on excluding peripheral vision the instability increases significantly up to the level of proprioceptive interference Without foamrubber no difference in stability could be established indicating that the level of proprioceptive interference here is lower than that of peripheral vision In the condition without foamrubber and with peripheral vision there is no chance that a perceptual conflict arises because with the nearby stationary contrasts in the visible periphery body movement is detected by both proprioception and vision.

The posturographic data support the hypothesis that physiological height vertigo is associated with a postural imbalance It was shown that lateral and fore–aft body sway as well as head sway increase non-linearly with increasing eye–object distance and that near by stationary contrasts in the periphery of the visual field reduce the body sway

The static stimulus condition of height or distance will not *per se* destabilize posture to an irresistible fall because of the redundancy in the control system The lack of an appropriate input is widely compensated for by the somatoreceptors and the labyrinths. However additional disturbances like wind or an unstable food support may result in serious problems in maintaining the upright position as subjects are apt to use the 'false' visual information in problematic circumstances Therefore patients with vestibular or somatosensory dysfunctions (e g polyneuropathy) are subjected to a particularly greater risk when exposed to height vertigo situations This also explains the results of Pogány (1958) who described different sensory deficits among his population of sufferers from height vertigo Because the postural behaviour of the subjects in the tilting room experiments and in the experiments with the varying eye–object distances was similar (large inter but small intra-individual differences) it is expected that subjects experiencing difficulties in the tilting room will also have difficulty in stabilizing posture when the critical eye–object distance is exceeded Thus the tilting room could serve to predict the individual susceptibility to physiological height vertigo The finding that under static conditions with eye–object distances exceeding 5 m vision does not seem to play any part in postural stabilization suggests that then larger retinal shifts (α) are required as hypothesized in paper I.

The posturographic data of the present study subserve the conclusions reached in paper I which were based on psychophysical data. Height vertigo can be minimized by

1 adjustment of the head to the gravitational vector when looking down since head tilt reduces postural stability by bringing the otoliths out of their optimal working range and therefore disproportionally enhancing the visual contribution to postural stabilization (pilot experiment)
2. providing nearby stationary cues in the periphery *of the visual field* since they provide peripheral vision with the necessary information to stabilize posture (Expt I)
3 avoiding free stance under height vertigo conditions because of the destabilizing role of vision under such circumstances (Expts I and II)

The physiological vertigo is primarily a result of the induced imbalance If the subject is lying down incongruity of the proprioceptive and visual information does not exist. The occurrence of physiological height vertigo is then unlikely This conclusion based on pos

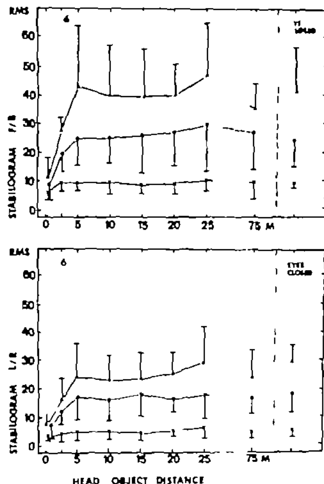


Fig 5 Mean RMS values (arbitrary units, $\pm 1\sigma$) of lateral and fore–aft stabilograms obtained at different eye–object distances (0.5 .5 5 10 15 20 25 and 75 m) both without (×) with single (O) and with double (Δ) foamrubber on top of the stabilometer The 6 subjects had a restricted visual field of 75° by means of a funnel attached to the head The eyes-closed' condition was tested as well

10 15 20 and 25 m The eyes-closed condition was tested as well These measurements were performed with the stabilometer without with single and with double layers of foamrubber For comparison these subjects were also tested at the 75 m condition of Expt I

As can be seen in Fig 5 the instability in the fore–aft direction is greater than in the left–right direction The results confirm the findings of Expt I in that the maximum RMS at an eye–object distance of at least 5 m are of the same order as the RMS obtained at the 75 m condition on the balcony Only the point of saturation shifted to the smaller distance of 5 m

DISCUSSION

Active movement of the seen environment may induce an apparent self motion sensation in the opposite direction followed by a postural reaction with a tendency to fall into the direction of pattern motion Such destabilizing visual effects have been demonstrated in several experiments (Dichgans et al 1976 Lee & Lishman 1975 Lestienne et al 1976) The tilting room however is the most natural stimulation since motion is about an axis at ankle height (Bles 1979 Bles et al 1977) Increasing tilt angles result in increasing visual-proprioceptive conflicts which permit an evaluation of multisensory interference on postural stability The results of Expt I with Tilting Room I reveal a level of proprioceptive interference which is relatively independent of the increasing amplitude of the sinusoidal tilt No vertigo was experienced by the subjects, indicating that these stimulus conditions were not particularly stressful It must also be emphasized that despite the fact that the subjects knew about the tilting of the room the body sway was influenced by the stimulus especially when standing on foamrubber This is consistent with the earlier findings obtained with Tilting Room I (Bles & de Wit 1976)

Expt II using Tilting Room II did not reveal a clear level of proprioceptive interference in the foamrubber conditions However in this experiment some of the subjects reported vertigo up to a complete loss of spatial orientation accompanied by distressing symptoms of optokinetic motion sickness

We believe that the foamrubber platform reducing the reliability of the joint receptors consequently reduces the redundancy of the reafferent sensory signals which are used for multisensory control of posture Then vertigo and postural destabilization are mainly due to the increased sensorial weight of the misleading visual signal which is contradicted only by the correct vestibular cues Thus the intensity of disequilibrium is a function of the magnitude of the mismatch and is increased if one of the sensory systems is eliminated such as with

Acta Otolaryngol 89 541–546 1980

SAC DECOMPRESSION FOR REFRACTORY LUETIC VERTIGO

Michael M Paparella Chong Sun Kim and Donald A Shea

From the Department of Otolaryngology University of Minnesota, Minneapolis USA

(Received August 14, 1978)

uetic hydrops can occur in both congenital l lues and otologic manifestations may be in- ble from Meniere disease Eight endolym- ecompression procedures were performed on ith refractory luetic vertigo Of those 3 had nd 3 acquired lues All cases had received ti-luetic medical therapy without any dis- rovement in the vertiginous attacks. In our 6 h luetic hydrops the main complaint was tory ertigo indeed disabling in most cases. ely all cases remained free from these severe the vertigo as controlled or eliminated in all ugh hearing was improved in one patient, ned not to improve generally speaking, as long-term postoperative follow-up W de- ethod of sac decompression and drainage and this procedure might be considered for pa- uncontrollable luetic vertigo who have not to adequate medical therapy

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Luetic hydrops may be seen in both congenital and acquired syphilis In 1966 Karmody & Schuknecht reported that 38% of 123 con genital syphilitics had a hearing loss most of whom had associated vestibular symptoms with episodic vertigo and diminished caloric responses They demonstrated a case of endo-lymphatic hydrops due to syphilitic osteitis of the otic capsule Similar findings were ob-served by Alexander (1928) and Mayer & Fra ser (1936) Perlman & Leek (195?) stated that endolymphatic hydrops secondary to invasion of the labyrinthine capsule may have some re semblance to the changes reported in Mem ere s disease This may account for the oc casional similarity of symptoms Schuknecht (1974) suggested that the inner ear reaction to lues is characterized by progressive endolym-phatic hydrops and degeneration of the mem-branous labyrinth resulting in rupture of the membranes due to overaccumulation of endo-lymph

Pulec (197?) reported that 7% of his patients with Meniere s syndrome complex were found to have a syphilitic etiology and Shea & Bow ers (1975) showed that 1 2% of the patients with a fluctuating hearing loss had a syphilitic origin. Severe episodic vertigo with nausea and vomiting in congenital syphilis is indistin-guishable from Meniere s disease and it may precede hearing loss by months or years and

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ZUSAMMENFASSUNG

Die Ergebnisse posturographischer Untersuchungen stimmen mit der Hypothese überein daß dem physiologischen *Höhenschwindel eine visuelle Destabilisation des auf*rechten Standes zugrunde liegt, wenn der Abstand zwischen Auge und stationären Umweltkontrasten kritisch groß wird 1) Sinusförmige Kippbewegungen eines künstlichen Raumes führen zu einem intersensorischen Wahrnehmungskonflikt zwischen visuellen vestibulären und somatosensorischen Meldungen mit optokinetischer Haltungsdestabilisierung in vorwärts–rückwärts oder seitlicher Richtung. 2) In einer naturlichen Umgebung nehmen die Schwankamplituden mit zunehmenden Auge-Objekt *Abstand* (bis zu 5 m) zu d h unter höhenschwindelauslösenden Reizbedingungen besteht eine reale Fallgefahr 3) Die Haltungsregulation kann verbessert werden a) durch eine natürliche aufrechte Kopfhaltung gegenüber dem Gravitationsvektor b) durch nahe stationäre Kontraste im peripheren Gesichtsfeld entsprechend der Dominanz der peripheren Retina für die dynamische Raumorientierung.

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Dr W Bles
E.N T Department
Free University Hospital
De Boelelaan 1117
Amsterdam
The Netherlands

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Luetic hydrops can occur in both congenital and acquired lues and otologic manifestations may be indistinguishable from Meniere disease Eight endolymphatic sac decompression procedures were performed on six patients with refractory luetic vertigo Of those 3 had congenital and 3 acquired lues. All cases had received anti-luetic medical therapy without any improvement in the vertiginous attacks. In our 6 patients with luetic hydrops the main complaint was refractory vertigo, indeed disabling in most cases. Postoperatively all cases remained free from these severe attacks and the vertigo was controlled or eliminated in all cases. Although hearing was improved in one patient it seemed not to improve generally speaking as shown in the long-term postoperative follow-up We describe the method of sac decompression and drainage and conclude that this procedure might be considered for patients with uncontrollable luetic vertigo who have not benefitted from adequate medical therapy

Luetic hydrops may mimic the symptoms of Meniere's disease However luetic hydrops is characterized by bilaterality earlier onset, and progressing symptoms The most disabling symptom of luetic hydrops is episodic attacks of vertigo

Eight endolymphatic sac decompression procedures were done on 6 patients with refractory luetic vertigo in an attempt to relieve the recurrent vertiginous attacks which were disabling and unresponsive to conservative treatment. We also hope to prevent further hearing impairment.

It is our intention here to describe this problem and to recommend consideration of surgical decompression of the endolymphatic sac in patients with luetic vertigo where extensive medical management has hitherto failed.

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may occur without hearing impairment In children with congenital syphilis the onset of deafness is not accompanied by marked vestibular symptoms such as is found in the adult group (Karmody & Schuknecht 1966)

The Tullio sign occurs more often in congenital syphilis (Schuknecht 1974) A positive fistula test without fistula or Hennebert s sign has been considered to be pathognomonic of congenital syphilis (Karmody & Schuknecht 1966 Perlman & Leek 1952) Nadol (1974) described fibrous adhesions between the footplate and membranous labyrinth and assumed this to be the pathological basis for Hennebert s sign This finding was common in congenital syphilis viral labyrinthitis and Meniere s disease Fiumara & Lessell (1970) analysed 271 patients with late congenital syphilis and 9 (3%) were found to have a hearing loss In these cases the onset was ushered by vertigo followed by hearing impairment

Hearing loss is reported to occur in about 25% to 38% of patients with congenital syphilis (Karmody & Schuknecht 1966) Tamari & Itkin (1951) reported the incidence of hearing loss in syphilis as 18% in late congenital 17% in early latent 25% in late latent 29% in asymptomatic syphilis and 80% in symptomatic syphilis

Patterson (1968) summarized the key to diagnosis of congenital luetic hearing loss as a high index of suspicion a sudden bilateral symmetrical sensorineural hearing loss without vertigo in childhood a sudden gradual asymmetrical sensorineural hearing loss with wide fluctuation and poor discrimination with or without vertigo in adult and positive serologic tests

Patients with luetic hydrops have traditionally been treated with extensive and if necessary prolonged medical therapy However in 1975 Shambaugh reported one patient who had congenital syphilis with labyrinthine hydrops who underwent an endolymphatic sac decompression procedure in his 105 cases of sac decompressions

In 1976 the senior author reported on 3 cases of luetic hydrops among his series of endolymphatic sac decompressions

We could find no previous specific report in the literature of surgical decompression for cases of refractory luetic endolymphatic hydrops

CLINICAL REPORTS

The senior author has performed endolymphatic sac decompression procedures in 1[illegible] cases of endolymphatic hydrops Eight ears 6 patients with refractory vertigo due to luet endolymphatic hydrops (5 female and o[illegible] male) are herein described

The method of decompression utilized h[illegible] been described previously and represents modification of the methods of Portmann an[illegible] House (1962) Three basic features of th[illegible] technique are as follows.

1 Wide exposure of the dura around th[illegible] endolymphatic sac which means all of Trau[illegible]mann s triangle including the infralabyrinthin[illegible] area and the adjacent margin of the later[illegible] sinus

2 Avoiding skeletonization of the posterio[illegible] semicircular canal Approaching the sac fron[illegible] behind leaving the canal imbedded in the soli[illegible] bone of contiguous facial buttress

3 Draining the sac with a T-tube into th[illegible] mastoid cavity fashioned out of Silastic sheet[illegible]ing which is held in place by the dura an[illegible] overhanging bony edge

Three patients (cases 1 2 3) aged 56 34 and 55 had late congenital lues with the first sign of interstitial keratitis at age 6 18 and 36 Hutchinson s teeth were found in case ? The remaining cases (4 5 6) aged 55 58 and 56 (male) had acquired lues Serologic tests were positive in blood and negative in CSF in all cases (Table I)

All cases had an audiovestibular symptom complex typical of ideopathic endolymphatic hydrops (Meniere s disease) The most disabling complaint at admission was long-standing incapacitating vertigo in all cases The vertigo was most often sudde

ble 1 *Clinical findings in 6 patients with refractory luetic vertigo*

					Blood		CSF	
e	Age & Sex	Race	First visit	Diagnosis & Findings	VDRL	FTA ABS	VDRL	VFT
S A V	56 F	W	Apr 1976	Congenital lues late interstitial keratitis at 6 years	–	2+	–	Bilateral hypoactivity
J J G	34 F	W	Apr 1970	Congenital lues late interstitial keratitis at 18 years. Hutchinson teeth	+		–	Bilateral hypoactivity
A.G.S	55 F	W	Nov 1968	Congenital lues late interstitial keratitis t 36 years. Cerebellar degeneration Fistula sign +		1+	–	Bilateral hypoactivity
Y E.	55 F	W	Aug 1973	Acquired lues, late latent at 19 years	+	4		Bilaterally reduced
C L	58 F	W	Jan 1973	Acquired lues, late neurosyphilis	+c	3		Bilateral hypoactivity
C K	56 M	W	Jan 1971	Acquired lues, late latent		4+	–	Bilateral hypoactivity

mpanied by nausea and vomiting Diplas was demonstrated in cases 5 and 6 d headache and pressure were present in 3 es. Hearing loss was first noticed in one r (aged 38 36 and 50 in congenital lues 3 50 54 and 43 in acquired lues) with nitus and pressure sensation and then in other ear with progressive deterioration of aring. Audiometry revealed sensorineural s in all cases with high tone loss at first amination in cases 1 3 and 6 and flat loss others Hearing thresholds fluctuated and ogressively deteriorated along with discrimition scores (14% in case 3 and 16% in se 5) SISI scores were very high in all ses including the unoperated ears and Besy tests showed Type II in both ears of se – Hearing loss was severe in one ear first examination in cases 1 3 and 6

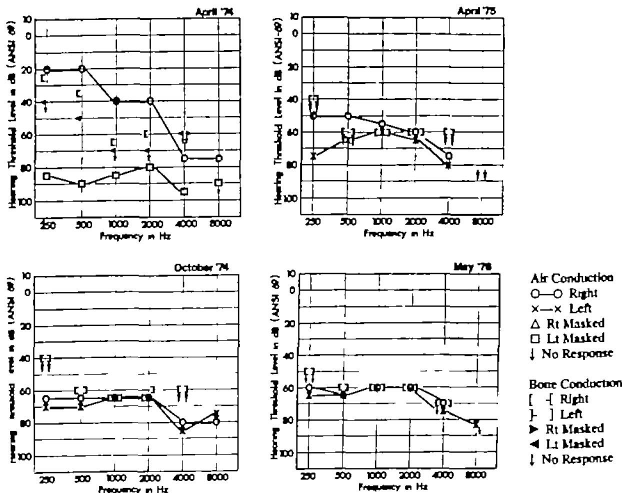
All cases received extensive anti-luetic atment Case showed improved hearing th an increased SRT (45 dB to 15 dB) and crimination score (92% to 100%) in her ht ear following an 8 day course of penilin and ACTH therapy However progressive deterioration of SRT (45 dB to 70 dB) and the discrimination score (88% to 50%) were noticed in the left ear without any improvement in the character or frequency of vertiginous episodes.

The postoperative period of follow-up varies between 3 and 8 years In some patients a sac decompression procedure in one ear (the worse ear) appeared to control the vertigo and the opposite side therefore did not receive surgery Although hearing was improved in one patient (25% increase in discrimination) it was preserved in all cases. In general progressive hearing loss developed over the years subsequent to the procedures

The following two representative patients are described in greater detail

Case 1

S A V white female age 56 a known congenital luetic with bilateral interstitial keratitis at age 6 had an onset of episodic whirling vertigo nausea and vomiting at age 16, occurring every few months lasting for a few hours A bilateral progressive hearing loss was first noticed 18 years ago in the left ear accompanied by continuous tinnitus. Hearing



Date	Apr 74		Oct 74		Apr 75		May 76	
Ear	SRT	PB	SRT	PB	SRT	PB	SRT	PB
R	30	100	70	60	55	60	60	84
L	95	CNT	75	68	65	68	60	84

Fig 1 Pre and post-operative successi e audiogr (case 1 S A V) Date of operation (*Right*) Octo 1975 (*Left*) June 1975

had recently worsened in her right ear which was her better ear (Fig 1) VDRL was nega tive and the FTA ABS test was 2+ in blood and VDRL was negative in CSF in June of 1974 She received a 10 day course of Bicil lin and Prednisone therapy in October 1974 Bithermal air caloric testing showed bilater al severely reduced vestibular function An audiogram revealed a bilateral sensorineural hearing loss with an SRT of 55 dB (R) and 65 dB (L) and discrimination scores of 60% (R) and 68% (L)

She was admitted with a diagnosis of con genital luetic hydrops with refractory vertigo and a 10-day course of Potassium Penicillin G and Solucortef was administered parenterally Her hearing thresholds remained unchanged on serial audiometric evaluation

Bilateral endolymphatic sac decompress was performed in June (L) and October 1975 Postoperatively she had a mild di feeling after her first operation but since second operation she has remained free fr vertigo and her hearing was stabilized with SRT of 60 dB with elevated discriminat scores (84% on both ears) at her most rec follow-up

This next patient was extremely incapa tated her vertigo was aggravated by riding a car She had to be practically carried i the clinic for initial visits

Case 4

V E white female age 55 had an onset periodic vertigo with accompanying nau and vomiting and fullness in her ears lasti

up to 3 hours at age 25. Her hearing began to deteriorate progressively in both ears starting 5 years ago It was fluctuating and progressive, worse on the right ear after a flu-like illness. She had syphilis at age 19 and was treated with Bismuth therapy She had also received a 70-day course of penicillin therapy 10 years ago

On admission (August, 1973) audiometric examination revealed a bilateral sensorineural hearing loss with an SRT of 60 dB (R) and 70 dB (L). Discrimination scores were 54% (R) and 58% (L) and SISI scores were 100% on both ears at 4 kHz. Caloric response was markedly reduced bilaterally with very slight subjective sensation in the left ear and none in the right ear VDRL was reactive and the FTA-ABS test was 4+ in blood and VDRL was negative in CSF Bicillin was given for 4 weeks with a diagnosis of late latent acquired syphilis. Subsequently additional medical therapy failed to control her symptoms and an endolymphatic sac decompression procedure was performed in September 1973 on the right ear and in February 1974 on the left ear

She is now rehabilitated Postoperatively she has had a mild sensation of pressure in her ear and slight imbalance However she has had no further vertiginous attacks and she is very happy being able to drive her car again.

DISCUSSION

Histopathologically the lesion of congenital syphilis cannot be differentiated from that of the acquired type It is characterized by progressive hydrops of the endolymphatic system, degeneration and atrophy of labyrinthine structures with rupture of the membranous labyrinth, causing severe incapacitating episodic vertigo accompanied by nausea and vomiting, fluctuating hearing loss and reduced speech discrimination. Tinnitus, loudness intolerance sensation of fullness in ears, and diplacusis are also present as in Meniere s disease Among those symptoms the most disabling symptom in luetic hydrops is refractory vertigo

Of our 6 patients, 4 cases initially presented with vertiginous episodes and 2 with cochlear symptoms (hearing impairment and tinnitus) However at the time of admission the patients chief complaint was incapacitating episodic vertigo which could not be controlled by conservative measures such as penicillin steroids erythromycin antiemetics sedatives or tranquillizers Three patients were diagnosed as late congenital syphilis with interstitial keratitis and Hutchinson s teeth (case 2) and the other 3 patients as acquired syphilis.

Intensive specific anti-luetic medical therapy may fail to prevent the onset of luetic vertigo deafness or to stabilize the disease process A hearing loss may occur despite adequate previous antibiotic or steroid therapy due to the variable response of the disease Our patients had received previous anti-luetic treatment without any significant effect in controlling incapacitating vertiginous episodes or progressing hearing impairment

In adequately treated syphilis viable spirochetes are occasionally found in eye CSF synovial fluid and lymph nodes and in the temporal bones of penicillin-treated congenital syphilitic deafness Spirochetes have been found in patients adequately treated with heavy metals Tamari & Itkin (1951) reported that despite administration of 9 million units of penicillin in 15 days, both hearing and vestibular function continued to deteriorate. The effective level of penicillin in vitreous humor spinal fluid or labyrinthine fluid against the spirochete is still open to question.

The patient who is allergic to penicillin has a serious problem in treatment South et al (1964) reported a case of a gravid woman treated with 15 g of erythromycin estolate over a 10-day period 2 months prior to delivery Her syphilitic infant died and the fetal level of erythromycin was only 1/5 to 1/20 of the maternal level Case 5 was allergic to penicillin and she was treated with erythromycin for

15 days prior to scheduling endolymphatic sac decompression

We considered parenteral streptomycin therapy to ablate the vestibular labyrinth in these patients This was done in one patient in this series and in 2 patients with intractable bilateral Meniere s disease without success Thus in our opinion the sac decompression seemed to be the procedure of preference

It is difficult to describe the physiological rationale of why a unilateral procedure would benefit the patient with bilateral disease These procedures were all performed for refractory vertigo This same observation was made in a study of certain patients with bilateral Meniere s disease From this small series of cases we have not determined that the procedure is beneficial for improving hearing in patients with luetic hydrops The procedure can be of unquestionable value to patients who have refractory vertigo however due to luetic hydrops where medical therapy has failed

ZUSAMMENFASSUNG

Lues bedingter Hydrops kann in der angeborenen und erworbenen Form auftreten und die otologischen Befunde *sind oft sehr schwierig von der Ménièrschen Krankheit zu* unterscheiden Acht endolymphatische Sack Decompressions-Operationen wurden an 6 Patienten mit refraktaren luetischen Vestibularisstörungen ausgeführt. Drei Patienten hatten Lues connatalis und drei die erworbene Form. Alle Patienten waren ausgiebig mit antiluetischen medizinischen Mitteln konservativ behandelt worden ohne jedoch irgenwelche Besserung der Schwindelanfälle aufzuweisen In unseren 6 Patienten mit luetischem Hydrops waren die Schwindelanfälle so intensiv und haufig daß sie in fast allen Fallen zur Berufsunfähigkeit führten Alle Patienten hatten postoperativ keine Vestibularisstorungen Das Hörvermogen war in einem Patienten besser und in den anderen Patienten war keine Horverbesserung im ‚Long-term follow-up aufzuweisen Unsere Operationsmethode zur endolymphatischen Sack Decompression und Drainage wurde veröffentlicht und kann in Patienten *mit* unkontrollierbarem luetischem Vertigo ausgeführt werden nachdem die extensive medizinische Behandlung erfolglos war

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Michael M Paparella M D
Department of Otolaryngology
Box 396 Mayo
University of Minnesota
Minneapolis Minnesota 55455
USA

Acta Otolaryngol 89: 547–554 1980

COMPUTER ANALYSIS OF HOARSENESS

Hisayoshi Kojima, Wilbur J Gould Anthony Lambiase and Nobuhiko Isshiki[2]

From the Lenox Hill Hospital New York, N Y USA

(Received May 15 1979)

ict The harmonic components in hoarse voice were ated from the noise components by using small atory computer The ratio of harmonics to noise N ratio, was calculated and compared with the audi- impression for 58 subjects. The calculated results ed definite correlation to the auditory impression. t is suggested that this would be useful method for antitative evaluation of hoarseness.

rseness is a symptom common to almost aryngeal ailments There have been many mpts to evaluate hoarseness as an aid to gnosis and these analyses have been sig- cantly advanced by the introduction of the nd spectrograph (Nessel 1960· Isshiki et 1966 Hiroto 1967 Yanagihara, 1967 ta et al. 1970· Rontal et al 1975)

A spectrogram of a normal sustained vowel ken with a narrow filter shows well de- loped harmonics as equally spaced horizon- l stripes while that of a hoarse voice pre- nts noise components as a cloudy shadow etween the harmonics. Harmonic com- ponents are replaced by noise components as e degree of hoarseness becomes more severe Fig. 1) If the acoustic energy of the har monics is calculated separately from that of he noise components, the degree of hoarse- ess can be determined as a ratio of har monics/noise or the signal/noise ratio

This study was undertaken to devise a meth- od by which to obtain the objective evaluation mentioned above through the use of a digital computer

Spectral Analysis

A periodic waveform can be represented as a sum of sinusoidal waves and each component sinusoid is called a harmonic of the waveform. Fourier Series Expansion is a basis for this harmonic analysis Its application, however is restricted to a periodic waveform

In speech analysis Fourier Integral For mula or Fourier Transformation which can be applied to an aperiodic waveform is preferred Since the acoustic speech waveform constant ly changes its characteristics as the articula- tors shift position it is practical to observe a short-term spectrum continuously

One way to obtain a short-term spectrum is to isolate a certain time interval (time window) from the waveform so that the waveform is zero everywhere outside the interval There is a relationship of inverse proportion between a time interval (Δt) and the bandwidth of the spectrum (Δw) as follows

$$\Delta t \cdot \Delta w = 1$$

From this equation it is concluded that a wide time window provides better frequency re solution while a narrow time window pro- duces better temporal resolution. Therefore the time interval should be determined by the purpose of the investigation

Since our aim in this study is to separate harmonics from noise components and cal- culate the signal/noise ratio it is desirable to set a wide time window and to use a tone

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Dr Kojima is on leave of absence from Department of Otolaryngology School of Medicine Kyoto University Kyoto Japan.

Director Voice Science Laboratory and Department of Plastic Surgery School of Medicine, Kyoto University Kyoto Japan.

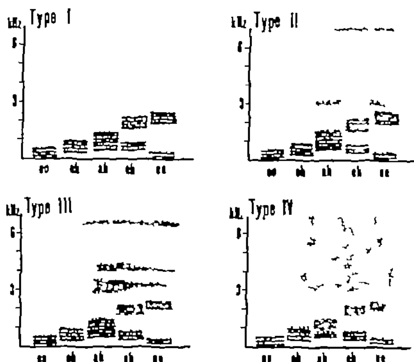


Fig 1 The four grades of hoarseness (from Yanagihara 1967)

which makes few articulatory changes during phonation such as a sustained vowel Since even a sustained vowel changes its frequency composition gradually because of vibrato or trend the interval of the time window has to be restricted and from our experience it is assumed to be 20–30 msec

Suppose that the voice waveform is analysed by Fourier Transformation utilizing a time window of 20–30 msec In this manner a continuous spectrum is obtained in which the individual harmonic components have a frequency bandwidth determined by the time window i e the narrower the time window the wider the bandwidth This spectrum is unusable for our study because the wide frequency bandwidth makes it difficult to separate the harmonic energy from the noise (Fig 2)

Fourier Series Expansion is used in this study because it offers a dispersal spectrum with good frequency resolution Since Fourier Series Expansion requires a periodic waveform the following idea was designed

Three pitch periods (*T*) were extracted from the voice waveform and repeated endlessly This results in the formation of a periodic wave which can then be subjected to analysis The display shows well defined harmonics and the noise energy can be easily recognized and

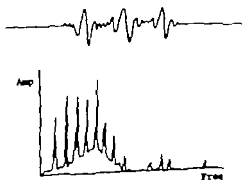


Fig 2 Waveform analysed by Fourier Transformation

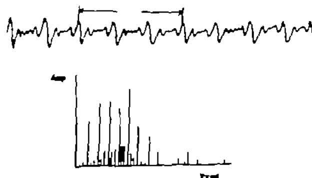


Fig 3 Periodic waveform analysed by Fourier Series Expansion.

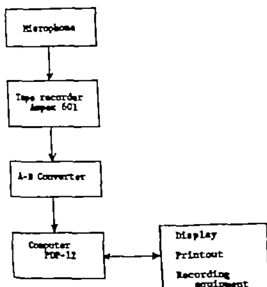


Fig. 4. Block diagram of the system.

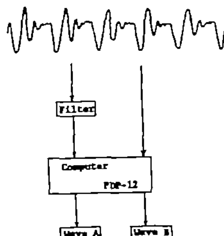


Fig. 5. Voice wave digitized without filter (B) and with filter (A).

extracted. It should be noted that the third harmonic on the display is actually the first harmonic of the voice waveform (Fig. 3).

METHOD

A block diagram of the system is shown in Fig. 4.

1. *Voice recording*

The subjects were 14 males and 14 females with no laryngeal or pulmonary disorders, and 20 males and 10 females with various laryngeal diseases resulting in various degrees of hoarseness (Table I).

A recording of each subject's voice was made in a sound-treated booth using a Electrovoice model 666 microphone and an Ampex model 601 tape recorder. A stable portion of the sustained vowel /a/ was used for the analysis.

Table I. *Types of voice disorders*

Type of disorder	N. of cases
Recurrent nerve paralysis	11 (2)
Atrophy	7 (3)
Polyp	7 (1)
Polypoid degeneration	1
Carcinoma	1
Nodule	1
Acute laryngitis	1
Mutational voice disorder	1 (1)

Numbers in the parentheses represent postoperative patients.

The voices on the magnetic tape were edited and arranged randomly in order to avoid listener judgment bias. Five persons who had experience in voice study were asked to judge the degree of hoarseness for each subject. The degree of hoarseness was rated as follows: 0=none, 1=slight, 2=fair, 3=extreme, and the scores were averaged for each subject.

2. *A/D conversion*

A 325 msec section of the voice signal was filtered with a 600 Hz low pass filter and digitalized (wave A). The same section of the voice signal was digitalized without filtering (wave B) (Fig. 5). The sampling rate of the A/D conversion was 22026 points per second. This rate was determined by the sampling program and both waves were stored on computer data tape.

3. *Pitch extraction*

Wave A was displayed on a scope and three pitch periods (T) were measured using a cursor. Fourier analysis was performed on the

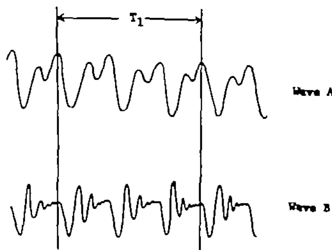


Fig 6 The calculation of T

corresponding pitch periods (T_1) of wave B (Fig 6)

4 *Fourier analysis*

If the three pitch periods (T_1) of wave B are repeated endlessly the result can be con sidered a periodic wave $f(t)$ whose pitch is T_1 where frequency is $F=1/T_1$ Therefore this wave $f(t)$ can be expanded into a series of sinusoidal waves whose pitch periods are T_1 $T_1/2$ $T_1/3$ T_1/n using Fourier Expansion as follows

$$f(t)=\frac{A_0}{2}+\sum_{1}^{\infty}\left(A_n \cos \frac{2n\pi}{T_1}t+B_n \sin \frac{2n\pi}{T_1}t\right)$$

$$=\frac{A_0}{2}+\sum_{1} X \quad \sin\left(\frac{2\pi}{T_1/n}t+E\right)$$

where

$$A_n=\frac{2}{T_1}\int_0^T f(t) \cos \frac{2n\pi}{T} t\, dt$$

$$B_n=\frac{2}{T_1}\int_0^T f(t) \sin \frac{2n\pi}{T_1} t\, dt$$

$$Xn=\sqrt{A_n^2+B_n^2}$$

Calculation

Since the fundamental pitch of the actual voice wave is $T_1/3$ (fundamental frequency $=3F$) harmonic components appear on the spectrum of $f(t)$ at $3F$ $6F$ $9F$ and between these harmonics are noise components In this study the wave $f(t)$ was expanded into sinusoidal waves with harmonic frequencies up to 5500 Hz, and this determined the value of n The signal/noise ratio of T_1 was calculated as follows

$$R_1(\text{dB})=10 \log_{10} \frac{S_1}{N_1}$$

where

$$S_1=X_3^2+X_6^2+X_9^2+X_{12}^2+$$

$$N_1=(X_1^2+X_2^2)+(X_4^2+X_5^2)+(X_7^2+X_8^2)+$$

This procedure was repeated on the next three pitch periods (T_2) and continued one after another (T_3 T_4 T_5 T_m) for 325 msec In order to obtain the average S/N ratio R was calculated as

$$R_a(\text{dB})=10 \log_{10} \frac{S_1+S_2+S_3+ \quad +S_m}{N_1+N_2+N_3+ \quad +N_m}$$

RESULTS

The R value for 28 normals ranged from 15 0 to 23 5 dB and Fig 7 shows the distribution The critical region of normal was 15 4 to 23.3 dB at a 5% significance level and no statistical difference was found between males and females

Table II presents the R values of patients voices along with the auditory impression for each voice Six subjects show values within

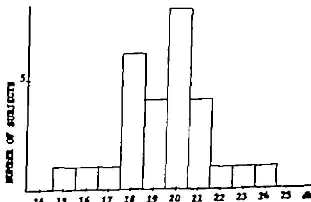


Fig 7 Histogram of normals.

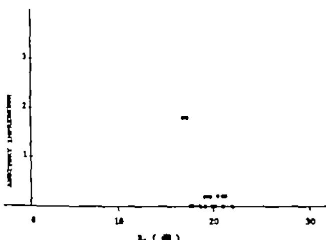


Fig. 8 Comparison of R_a value to listener judgment.

the critical region of normal. The overlap is not unexpected because in this study the pathologic group consisted of different stages of various disorders including postoperative voices. The breakdown of pathologies of the 6 subjects is listed below and includes the operative procedures.

1 case recurrent nerve paralysis (Thyroplasty I)
1 case polyp (Polypotomy)
? cases, atrophy (Thyroplasty I used in 1 case)
1 case nodules
1 case mutational voice disorder (Thyroplasty III)

Table II *The R_a value and auditory impression of pathologic voices*

Subject no	R_a (db)	Auditory impression
1	12 6	..0
2	3 9	2 4
3	12 5	2 2
4	10 1	2 0
5	4 7	2 8
6	1 5	3 0
	16 8	0 8
8	13 8	2 0
9	1 7	2 0
10	17 3	1.8
11	17 6	1 8
12	20 3	0 6
13	3 5	1 4
14	0.9	3 0
15	11 3	2.4
16	4 5	3 0
17	13 9	2 4
18	2 5	3 0
19	18 3	1 6
20	10 5	6
21	15 2	2 0
22	6.4	3 0
23	3 7	3.0
24	12 1	2 0
25	10 9	1 6
26	7 8	2 8
27	16 3	2 0
28	9 3	2 0
29	11 1	2 0
30	18 7	0 6

In order to compare each R value with its auditory impression more clearly the score of the listener judgment was plotted against the R value for both normal and pathologic voices in Fig. 8. It was noted that there is a definite correlation between the two parameters. The calculated correlation coefficient was −0 868 and significant at the 1% level. Figs. 9, 10 and 11 present sonagrams for slight, moderate and severe hoarseness. The R_a values were 15.2 dB (case 21), 11 1 dB (case 29) and 4,5 dB (case 16) and these values correspond well to the visual impressions of the sonagrams.

Cases 9 and 13 show a slight deviation from the general trend. The sonagrams of these voices (Figs. 12 and 13) present sudden changes in the voice quality. Auditory impression is considered as the impression of voice over time while our method has a limitation in that it can store only 325 msec samples of

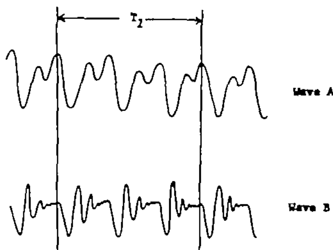


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This procedure was repeated on the next three pitch periods (T_2) and continued one after another (T_3 T_4 T_5 T_m) for 325 msec In order to obtain the average S/N ratio R_a was calculated as

$$R_a(\text{dB})=10\log_{10}\frac{S_1+S_2+S_3+\quad+S_m}{N_1+N_2+N_3+\quad+N_m}$$

RESULTS

The R value for 28 normals ranged from 15 0 to 23.5 dB and Fig 7 shows the distribution The critical region of normal was 15 4 to 23.3 dB at a 5% significance level and no statistical difference was found between males and females

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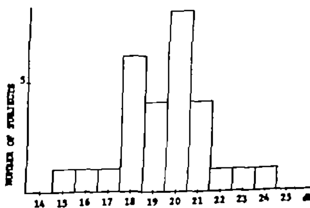


Fig 7 Histogram of normals.



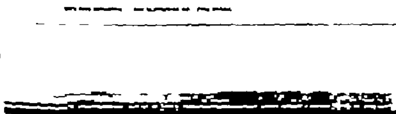


Fig 12 Sudden change in voice quality (case 9).

Fig 13 Sudden change in voice quality (case 13).

tion or vocal shimmer was analyzed by Koike (1969), von Leden & Koike (1970) and Kitajima (1976) The previously mentioned investigators using computer analyses, have been successful in evaluating a rough quality which is related to an irregularity of the fundamental cycle

When combined with the previous methods, the calculation of the S/N ratio of hoarse voice can distinguish a breathy voice from a rough voice Furthermore the S/N ratio of hoarse voice obtained by the present method appears to be a quantitative indicator of the degree of hoarseness thereby providing objective information on the effectiveness of individual treatment.

ZUSAMMENFASSUNG

Die harmonischen Bestandteile in der heiseren Stimme wurden von den Lärm-Bestandteilen durch einen kleinen Laboratoriums-Computer abgesondert. Das Verhältnis von Harmonie zu Lärm oder H/L-Verhältnis (S/N Ratio) wurde berechnet und verglichen zu dem Gehöreindruck von 91 Personen. Die kalkulierten Resultate zeigen einen Zusammenhang zum Gehöreindruck und es wurde vorgeschlagen, Daß dieses eine hilfreiche Methode sein würde für eine quantitative Bewertung von Heiserkeit.

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Fig 9 Sonagram of slight hoarseness (R_a = 15.2 dB).



Fig 10 Sonagram of moderate hoarseness (R_a = 11 1 dB)

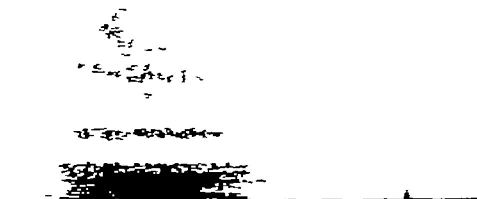


Fig 11 Sonagram of severe hoarseness (R_a = 4.5 dB).

the voice This may be assumed to be a reason why this deviation occurred

DISCUSSION

There have been many attempts to establish objective parameters which represent the degree of hoarseness but the term hoarseness includes a wide range of deviation from the normal voice Some attempts to quantify the measurement of hoarseness have dealt with auditory impression Isshiki et al (1966 1970) using Osgood's semantic differential method introduced a technique to classify the auditory impression of hoarse voice As a result of his analysis he found that hoarse voice has four factors Rough (R) Breathy (B) Asthenic (A) and Degree (D)

Further investigation has been done on the acoustic features of hoarseness by Hiroto (1967) using a sonagraph It was revealed that the breathy quality is characterized by a large degree of noise components and a decrease in harmonic component. The rough quality is distinguished by a great cycle to-cycle fluctuation in the fundamental frequency

In an attempt to obtain objective parameters which represent the degree of hoarseness pitch perturbation was measured by Lieberman (1961) Iwata & von Leden (1970) Hiki (1966) and Kitajima (1975) Amplitude varia-

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LYMPHOCYTE ATPase ACTIVITY IN PATIENTS WITH CARCINOMA OF THE LARYNX

A Follow-up Study on 45 Patients

J Ellegaard and A Toft

From the University Departments of Medicine and Clinical Haematology Aarhus Amtssygehus, and Otolaryngology Aarhus Kommunehospital Aarhus Denmark

(Received April 19 1979)

Abstract Mitochondria-related Mg ATPase activity was assayed in lymphocytes isolated from the blood in 45 patients with epidermoid carcinomas of the larynx. Increased activity was found in 11 patients and normal or subnormal activity in the remaining cases No correlation could be demonstrated between the magnitude or the spreading of the malignancy as recorded by the TNM classification and the ATPase activity In 36 patients determinations were carried out repeatedly during period from 6 to 20 months following therapy and after 3 years final follow-up on all patients was done. All patients received high-voltage radiotherapy and in 7 patients additional partial or total laryngectomia was carried out, one patient had bilateral neck dissection After radiotherapy the ATPase activity decreased significantly in 17 patients while it either remained normal or increased temporarily in the rest. No correlation was found between the course of the disease and the initial ATPase activity or the pattern of variation in ATPase activity following therapy Contrary to what resulted from preliminary study it is concluded that determination of lymphocyte ATPase activity is of no diagnostic value in patients with laryngeal carcinoma, nor does it offer any prognostic help in determining which patients may relapse or develop metastatic disease

Increased mitochondrial lymphocyte ATPase activity has previously been found in patients with various malignancies (Ellegaard & Dimitrov 1972a 1972b Dimitrov & Ellegaard 1972, Dornand et al 1976) Preliminary results from determination of lymphocyte ATPase activity in 10 patients with laryngeal carcinoma (Ellegaard et al 1975c) have shown that half of the patients investigated had increased lymphocyte ATPase activity compared with normals and after radiotherapy this activity was normalized

In the present study we have expanded these preliminary observations on larynx carcinoma to comprise a larger number of patients at different clinical stages of the disease *Repeated determinations of the lymphocyte* ATPase activity were performed during a mean observation period of 30 months in order to establish a possible relationship between the lymphocyte ATPase activity and relapsing or metastasizing neoplasia. The results have not confirmed any relationship between the magnitude or spreading of the disease and the lymphocyte ATPase activity

MATERIALS AND METHODS

The study comprises a control group of 40 normal healthy subjects of both sexes (20 males, age: 18–70 years and 20 females age: 21–86 years) and a group of 45 patients with carcinoma of the larynx 4 females and 41 males 34 to 86 years of age (average age 63 years) All patients had histologically *proven* epidermoid carcinomas of which 13 were supraglottic and 32 glottic According to the UICC classification 6 of the patients with supraglottic tumours belonged to stage T 1 to stage T_2 5 to stage T and 1 to stage T Of the 32 patients with glottic tumours 22 belonged to stage T 9 to stage T_2 and 1 to stage T One of the patients with supraglottic tumours had regional lymph node metastases

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Hisayoshi Kojima M.D
Vocal Dynamics Laboratory
Lenox Hill Hospital
100 East 77 Street
New York N Y 10021
U.S.A

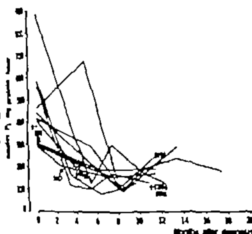


Fig. 1. Eleven patients with initially increased ATPase activity. In 10 cases the activity decreased to normal levels after radiotherapy. Four patients died within the control period and their individual survival time (in months) indicated by figure in brackets. *ND*: Neck dissection. *R*: Remaining tumour mass or relapse. *M*: Metastatic disease.

eased lymphocyte ATPase activities. Individual values are shown in Table I, in which e staging of the tumours and the clinical sponse to treatment in these patients also pear. Following radiation therapy the increased ATPase activities were normalized in l these patients (Fig. 1) except in one, name 'a patient who died before the first control he lymphocyte ATPase activity was normalized also in ? of the patients despite persisting tumour mass in one and bilateral lymph node metastases in the other.

In 7 patients (Table II) the ATPase activity rose temporarily from an initial normal level to significantly increased values (Fig. 2) after radiotherapy. This observation was not in any case related to the clinical course of the disease. Only 1 of these patients died from relapse and metastases within the control period (29 months after therapy).

The remaining 27 patients (Tables III and IV) also had normal ATPase activities before radiotherapy and the activity remained normal in 19 of these patients who were followed repeatedly after treatment (Fig. 3). In no case did the lymphocyte ATPase activities seem to be correlated to the clinical course of the disease. Three of the patients who had a remaining tumour mass after radiotherapy were operated on and they finally succumbed to their disease (Table III). Two other patients died from causes not related directly to their neoplastic disease.

After a mean observation period of 30 months 36% of the patients had relapsed among those having an initially increased ATPase activity, whereas only 15% relapsed among the patients with a normal ATPase activity before therapy. However this difference is not significant ($p>0.2$, Fisher's exact test).

A clinical evaluation of the patients after

able II. *Seven patients with initially normal lymphocyte ATPase activity but in whom the ctivity later inc eased after therapy (Fig. 2)*

. 3-year follow-up after therapy one patient (14 ?) had died of tumour relapse and metastases
TL: Total laryngectomy. PL: Partial laryngectomy.

atients	Age (yrs)	Sex	Tumour localization	Tumour stage	Effect of treatment	Operation	Length of observation period (months)	Relapse at the end of observation	Initial ATPase activity (nmoles P_i/mg/h)
TAMN	63	♂	Glottis	$T_3N_0M_0$	Incomplete	TL	42	–	153
EOK	52	♂	Supraglot	$T_{1a}N_0M_0$	Complete	TL	29	+	153
DC	46	♂	Glottis	$T_{1a}N_0M_0$	Incomplete	PL	38	–	46
GEB	77	♂	Glottis	$T_{1a}N_0M_0$	Complete		43		198
TTL	52	♂	Glottis	$T_{1a}N_0M_0$	Complete		42	–	86
JKB	86	♂	Glottis	$T_2N_0M_0$	Complete		44	–	140
AA	65	♂	Glottis	$T_{1a}N_0M_0$	Complete		36	–	263

Table I *Eleven patients with initially increased lymphocyte ATPase activity*

In 10 patients the ATPase activity permanently decreased to normal levels after therapy (Fig. 1). At a [illegible] year follow up 4 patients (36%) had died from tumour relapse or metastases

TL Total laryngectomy BLND Bilateral lymph node dissection

Initials	Age (yrs)	Sex	Tumour localization	Tumour stage	Effect of treatment	Operation	Length of observation period (months)	Relapse at the end of observation	Initial ATPase activity (nmole P_i/mg/h)
RPS	71	♂	Supraglot	$T_4N_0M_0$	Incomplete	–	6	+	383
DP	56	♀	Glottis	$T_{1a}N_0M_0$	Complete	–	37	–	884
KAJ	73	♂	Glottis	$T_2N_0M_0$	Complete	–	19	+	291
BAVR	59	♂	Glottis	$T_1N_0M_0$	Complete	–	4	–	419
NPKK	82	♂	Glottis	$T_3N_0M_0$	Complete	–	38	–	444
JVJ	58	♂	Glottis	T[illegible]N_0M_0	Complete	TL	25	+	588
HD	73	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	38	–	301
GJ	58	♂	Supraglot	T[illegible]N_2M_0	Incomplete	BLND	42	–	291
EP	48	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	39	–	467
KDK	58	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	37	–	564
ASAP	68	♂	Supraglot	T_3M_0N	Incomplete	–	4	+	409

upon presentation but none of the patients with glottic tumours had lymph node metastases None of the patients were included in our previous study (Ellegaard et al 1975*c*) All the patients were treated with high-voltage irradiation (6 000 rad) given within a period of 6 weeks The result of the irradiation therapy is recorded as complete when the primary tumour was no longer visible or palpable and no signs of metastases had appeared at a clinical follow-up examination 6–12 weeks later

Before radiation therapy blood samples were drawn from all patients for determination of the ATPase activity in circulating lymphocytes and in 37 patients blood samples were repeatedly drawn and assayed for lymphocyte ATPase activity after various intervals following the treatment All cases were finally evaluated at a 3 year follow up and all data accumulated were analysed

The lymphocytes were isolated from 20 ml heparinized venous blood essentially as described previously (Ellegaard & Dimitrov 1972*a*) Passing of the blood through a glass bead column was omitted however in order to avoid depletion of lymphocyte subpopulations e g of B-lymphocytes Furthermore phagocytes were removed from the primarily harvested suspension of mononuclear cells b the carbonyl iron method (Shah & Dickso 1974) Possibly contaminating erythrocyte were lysed by a 5 min exposure of the ce suspension to a 0 84% ammonium oxala solution at 37° The cells were suspended cold 0 25 M sucrose (10^6 cells per ml) an sonicated for 2×15 sec under continuous coo ing The supernatant of the resultant hom genate was assayed for ATPase activity b the same method as previously used witho modifications (Ellegaard & Dimitrov 1972*a*) Liberation of inorganic phosphate (P_i) fro Tris-ATP during incubation for one hour a 37° was determined and the results were co rected for endogenous lymphocyte P_i and non enzymatic P release during the incubation Double incubations and double determina tions on each incubation were used through out The final results are expressed as nmole P_i liberated per mg protein of the supernatan per hour

RESULTS

The normal controls had a mean lymphocyte ATPase activity of 210±70 (2 S D) nmole P_i/mg/h

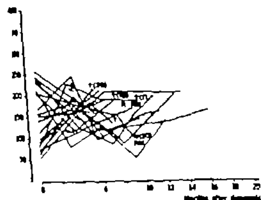
Eleven patients (25%) had initially in-

ble IV Eight patients with initially normal lymphocyte ATPase activity and in whom only e ATPase determination was carried out

: follow-up 3 years after therapy one patient (12 %) had died from tumour relapse and metastases

rtals	Age (yrs)	Sex	Tumour localization	Tumour stage	Effect of treatment	Operation	Length of observation period (months)	Relapse at the end of observation	Initial ATPase activity (nmoles P$_i$/mg/h)
P	59	♂	Supraglot	$T_2N_0M_0$	Complete	–	25	–	170
APB	64	♂	Glottis	$T_1N_0M_0$	Incomplete		10	+	105
EFD	59	♀	Glottis	$T_{1b}N_0M_0$	Complete		26	–	165
AK	54	♂	Glottis	$T_{1a}N_0M_0$	Complete		26	–	138
LAL	68	♂	Glottis	$T_{1a}N_0M_0$	Complete		26	–	195
SEJ	54	♂	Glottis	$T_1N_0M_0$	Complete	–	25	–	104
FC	65	♂	Glottis	$T\ N_0M_0$	Complete		28	–	169
SAL	51	♂	Glottis	$T_1N_0M_0$	Complete	–	26	–	58

Tumour cell specific sensitization of the killer cells could be due to circulating tumour cell associated antigens but non-specific killing may also be effective (Pross & Baines 1977) The energy needed for the cytotoxic potential details of which are still not understood, could be provided by hydrolysis of ATP by mitochondrial Mg ATPase As a matter of fact it has previously been shown that a mitochondrial ATPase activity is present in human normal lymphocytes in the blood (Ellegaard & Dimitrov 1973) and that this ATPase activity is increased in patients with various neoplastic tumours including carcinoma of the larynx (Ellegaard & Dimitrov 1972*a* 1972*b* Dimitrov & Ellegaard 1972 Ellegaard et al 1975*a* 1975*b* 1975*c*) Contrary to what was believed from the beginning it seems to be the B-lymphocytes which carry the highest ATPase activity in unstimulated normal human lymphocytes isolated from the blood (Kragballe & Ellegaard, 1978) and elevated ATPase activity is also found in lymphocytes from patients with B-lymphocyte proliferative diseases like chronic lymphocytic leukaemia (Ellegaard, 1979)



e 3 Nineteen patients with initially normal or subnormal ATPase activity After radiotherapy the ATPase activity decreased in 7 patients, while it increased in the 1 other cases Five patients died within the control period 3 from relapse or metastases, the 2 from causes not related to their malignant disease *PL* Partial laryngectomy *TL* Total laryngectomy *R* Relapse *RM* Relapse

The normal range for the lymphocyte ATPase activity is higher in the present study than in the previous investigation (Ellegaard et al 1975*c*) This is most likely due to alterations in the methods omitting removal of the sticky populations of cells by passing the mononuclear cell suspensions through glass-bead columns. The present study has confirmed that the ATPase activity of circulating lymphocytes is increased in a number of patients with carcinoma of the larynx and that the ATPase activity in these cases decreases to normal levels after radiotherapy Increased protein RNA and DNA synthesis has been demonstrated in human lymphocytes transformed

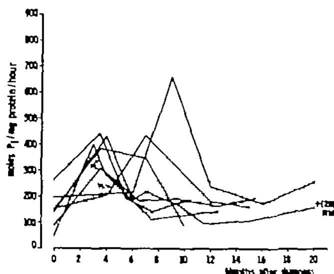


Fig 2 Seven patients with initially normal ATPase activity After radiotherapy the activity rose temporarily but after several months the activity was again normalized in all patients. One patient died from tumour relapse and metastases (after 29 months) *PL* Partial laryngectomy *TL* Total laryngectomy *RM* Relapse and metastases

radiotherapy did not correspond to the ATPase activity level recorded in the following control period In the 7 patients where the treatment was recorded as incomplete the mean of the highest ATPase activities was 264 nmoles/mg/h in comparison with 259 in 29 patients where the result of the treatment was deemed complete Similarly no difference was found between the individual mean value of all ATPase activities determined within the control period in patients belonging to either one of these groups

It was not possible through this study to demonstrate any relationship between the survival time and either the initial ATPase activity or the average ATPase activity in the control period as most patients were still alive at the follow up 3 years after therapy

DISCUSSION

Killing of neoplastic cells in vivo can be carried out by various cellular mechanisms which ultimately include direct action by close cell to cell contact between activated lymphocytes or macrophages and the malignant target cell (Hellström et al 1971 Perlman et al 1972).

Table III *Nineteen patients with initially normal ATPase activities and in whom this activity remained normal (Fig 3) during a control period varying from 7 to 20 months*

At a follow-up after 3 years, 3 patients (16%) had died from tumour relapse and metastases
TL Total laryngectomy PL Partial laryngectomy

Initials	Age (yrs)	Sex	Tumour localization	Tumour stage	Effect of treatment	Operation	Length of observation period (months)	Relapse at the end of observation	Initial ATPase activity (nmoles P_i/mg/h)
SPH	70	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	8	–	260
SGJ	48	♂	Supraglot	$T\ N_0M_0$	Incomplete	PL	20	+	208
TC	50	♂	Supraglot	$T_2N_0M_0$	Incomplete	TL	10	+	208
NREM	70	♂	Supraglot	$T\ N_{1a}M_0$	Incomplete	TL	7	+	85
JKK	66	♂	Supraglot	$T_2N_0M_0$	Complete	–	32	–	95
HD	46	♂	Supraglot	$T_3N_0M_0$	Complete	–	34	–	66
LVH	82	♀	Supraglot	$T\ _bN_{1a}M_0$	Complete	–	39	–	161
JAM	57	♂	Supraglot	$T\ _bN_0M_0$	Complete	–	31	–	76
EDP	34	♀	Supraglot	$T_{1a}N_0M_0$	Complete	–	35	–	244
AMN	73	♂	Glottis	$T_2N_0M_0$	Complete	–	23	–	177
KAJ	71	♂	Glottis	$T\ N_0M_0$	Complete	–	37	–	56
KGT	75	♂	Glottis	$T_2N_0M_0$	Complete	–	28	–	38
HRJ	61	♂	Glottis	$T\ _aN_0M_0$	Complete	–	38	–	21
JCC	63	♂	Glottis	$T\ N_0M_0$	Complete	–	29	–	115
HL	68	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	39	–	46
SH	70	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	33	–	147
RFK	66	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	34	–	155
HJHC	63	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	31	–	100
GOA	65	♂	Glottis	$T_{1a}N_0M_0$	Complete	–	30	–	63

ble IV *Eight patients with initially normal lymphocyte ATPase activity and in whom only e ATPase determination was carried out*

: follow-up 3 years after therapy one patient (12 ♀) had died from tumour relapse and metastases

ents	Age (yrs)	Sex	Tumour localiza-tion	Tumour stage	Effect of treatment	Opera-tion	Length of ob-serva-tion period (months)	Relapse at the end of observa-tion	Initial ATPase activity (nmoles P_i/mg/h)
'	59	♂	Supraglot	$T_2N_0M_0$	Complete	–	25	–	170
FB	84	♂	Glottis	$T_2N_0M_0$	Incomplete		10	+	105
FD	59	♀	Glottis	$T_{1a}N_0M_0$	Complete		26	–	165
K	54	♂	Glottis	$T_{1b}N_0M_0$	Complete	–	26	–	138
AL	68	♂	Glottis	$T_{1a}N_0M_0$	Complete		26	–	195
EI	54	♂	Glottis	$T_2N_0M_0$	Complete		25	–	104
;	65	♂	Glottis	$T_1N_0M_0$	Complete	–	28	–	169
AL	51	♂	Glottis	$T_1N_0M_0$	Complete	–	26	–	58

tumour cell specific sensitization of the killer cells could be due to circulating tumour cell associated antigens, but non-specific killing may also be effective (Pross & Baines, 1977) The energy needed for the cytotoxic potential details of which are still not understood could be provided by hydrolysis of ATP by mitochondrial Mg-ATPase As a matter of fact it has previously been shown that a mitochondrial ATPase activity is present in human normal lymphocytes in the blood (Ellegaard & Dimitrov 1973) and that this ATPase activity is increased in patients with various neoplastic tumours including carcinoma of the larynx (Ellegaard & Dimitrov 1972*a* 1972*b* Dimitrov & Ellegaard 1977 Ellegaard et al 1975*a* 1975*b* 1975*c*) Contrary to what was believed from the beginning it seems to be the B-lymphocytes which carry the highest ATPase activity in unstimulated normal human lymphocytes isolated from the blood (Kragballe & Ellegaard 1978) and elevated ATPase activity is also found in lymphocytes from patients with B-lymphocyte proliferative diseases like chronic lymphocytic leukaemia (Ellegaard 1979)

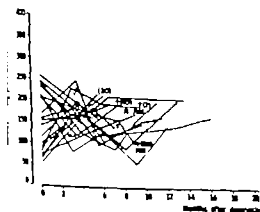


Fig. 3 Nineteen patients with initially normal or subnormal ATPase activity After radiotherapy the ATPase activity decreased in 7 patients, while it increased in the 11 other cases Five patients died within the control period, 3 from relapse or metastases, the 2 from cancers not related to their malignant disease *PL* Partial laryngectomy *TL* Total laryngectomy *R* Relapse *RM* Relapse and metastases.

The normal range for the lymphocyte ATPase activity is higher in the present study than in the previous investigation (Ellegaard et al 1975*c*) This is most likely due to alterations in the methods omitting removal of the sticky populations of cells by passing the mononuclear cell suspensions through glass-bead columns. The present study has confirmed that the ATPase activity of circulating lymphocytes is increased in a number of patients with carcinoma of the larynx and that the ATPase activity in these cases decreases to normal levels after radiotherapy Increased protein RNA and DNA synthesis has been demonstrated in human lymphocytes transformed

into a blastic stage by exposure to tumour antigens (Jehn et al 1970) or phytohaemagglutinin (Craddock et al 1971) The latter also leads to increased synthesis of phosphoproteins decreased intracellular concentration of ATP (Kleinsmith et al 1966) and *increase* in the mitochondrial ATPase activity (Ellegaard & Dimitrov 1972*a* Dimitrova 1977) Also we have demonstrated that *exposure* of normal lymphocytes to various concentrations of antilymphocyte globulin *stimulates* the ATPase activity *proportionally* (Kragballe & Ellegaard 1977) Stimulation of the lymphocyte membrane receptors by antigen–antibody complexes leading to nuclear activation increased DNA synthesis and increased cytotoxicity against target cells was demonstrated in 1969 (Möller) Similar biochemical events could result from stimulation of circulating lymphocytes by tumour associated antigens

However a direct link between activation in neoplastic disease of the lymphocyte membrane receptors of which Fc-receptors seemed to be essential for the killing effect (Pross & Baines 1977 Galili & Schlesinger 1978 Kristensen & Langvad 1978 Lotzova & McCredie 1978) and the mitochondrial ATPase activity has not yet been demonstrated The synthesis and *maintenance* of the lymphocyte membrane receptors is an energy requiring process (Berke & Gabison 1975 Galili & Schlesinger 1975 Buschkin et al 1975) which is also dependent on the cyclic AMP system (Chisari & Edgington 1974 Grieco et al 1976) In a previous study we found significantly higher ATPase activity in a B-cell enriched lymphocyte suspension having *more* Fc receptors (Kragballe & Ellegaard 1978) but in the present investigation we did not intend to measure the ATPase of different lymphocyte subpopulations

The present study has demonstrated that determination of the lymphocyte ATPase activity in patients with laryngeal carcinoma does not add another diagnostic tool to the existing ones as significantly elevated ATPase activities were only found in 25 % of all the patients in the *untreated* stage and as no correlation could be found between the tumour stage and the ATPase activity Also a decrease in the ATPase activity to normal levels was recorded in two patients despite a persisting considerable tumour mass and a rising ATPase activity was not invariably consistent with relapse or metastasizing within a control period of 30 months

Thus the clinical value of ATPase determinations in patients with carcinoma of the larynx seems to be very slight and therefore cannot be recommended

ACKNOWLEDGEMENTS

The authors are indebted to Mrs Kate Sommer and Mrs Lise Hauge for expert technical assistance The work was supported by grants from the Danish Medical Research Council (grants 51 –4113 6715 8198).

ZUSAMMENFASSUNG

Die Aktivität der Mitochondrien-Mg-ATPase wurde in Lymphozyten die aus dem Blut von 45 an Epidermoidcarcinom des Kehlkopfes erkrankten Patienten isoliert wurden, bestimmt Bei 11 Patienten wurde erhöhte oder subnormale Aktivität gemessen. Große und Malignitätsausdehnung der Tumoren wurde gemäß der TNM Klassifikation registriert· die Ergebnisse ließen sich jedoch nicht mit der ATPase Aktivität korrelieren Bei 36 Patienten wurden Enzymbestimmungen in einem Zeitraum von 6 bis 20 Monaten nach Therapiebeendigung durchgeführt, und nach 3 Jahren wurde eine abschließende alle Patienten umfassende Nachuntersuchung vorgenommen. Alle Patienten erhielten high-voltage Radiotherapie und 8 Patienten wurden zusätzlich operiert (partielle oder totale Neck-dissektion) Nach Beendigung der Strahlentherpaie nahm bei 17 Patienten die ATPase Aktivität signifikant ab während bei den restlichen Patienten normale oder vorübergehend ansteigende Werte gemessen wurden. Es konnte keine Korrelation zwischen Krankheitsverlauf und initialer ATPase-Aktivität nachgewiesen werden, es gelang auch nicht eine Regelmäßigkeit des Variationsmusters der ATPase Aktivität nach Therapieabschluß aufzuzeigen. Im Gegensatz zu den Ergebnissen einer eigenen Vorstudie sind wir zu dem Schluß gekommen, daß die Messung der Lymphozyten-Mg-ATPase Aktivität weder zur Sicherung der Diagnose des Larynxcarcinoms beitragen kann noch prognostische Aussagen hinsichtlich Rezidivhäufigkeit oder Metastasierung zuläßt

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J Ellegaard M D Ph.D
Dept Med H ematol II
Aarhus Amtssygehus
DK 8000 Aarhus C
Denmark

Acta Otolaryngol 89 562–569 1980

AMYLOIDOSIS OF WALDEYER S RING

A Clinical and Ultrastructural Report

M Beiser [1] G Messer [2] J Samuel [1] B Gross[2] and E Shanon[1]

From the Department of Otolaryngology and Laboratory of Electron Microscopy Ichilov Medical Center Tel Aviv and Sackler School of Medicine University of Tel Aviv Israel

(Received May 29 1979)

Abstract Amyloidosis of the tonsil is a rare condition and amyloidosis of Waldeyer s ring has not been previously reported. The present study describes a case of amyloidosis of the tonsil nasopharynx and base of tongue in a 35-year-old patient in otherwise good health and without clinical symptoms The possibility of systemic amyloidosis was excluded by clinical examination and biopsy of rectal and buccal mucosae Electron microscopy showed the presence of typical amyloid fibrils Large masses of the fibrils were closely surrounded by cells which appeared to be phagocytic The ultrastructure of these cells is described This case is considered to be a unique type of organlimited amyloidosis of Waldeyer s ring

The term amyloid refers to a pathologic proteinaceous substance deposited in the extra cellular compartment of various organs and tissues (Cohen 1967 Glenner & Page 1976) While amyloid deposits have been shown to comprise a heterogeneous group of substances they are commonly defined by their birefringence under polarized light after Congo red staining and their characteristic fibrillar structure by electron microscopy Studies by X ray diffraction have indicated that such amyloid fibrils contain a β-pleated configura tion (Glenner & Page 1976 Cohen et al 1978 Pras & Gafni 1978)

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chiae and ecchymoses which tend to bleed easily. Localized deposits of amyloid are common in oral plaques, diffuse thickenings or translucent papillas. Amyloid infiltration has been reported in certain odontogenic tumors (Vickers et al. 1965). The deposition of amyloid frequently precedes the calcification in the tumor.

There have been only a few reports of amyloid deposits in the tonsils (Mutschler 1933, Chiari 1937, Eriksen 1970). In these, the presenting symptoms varied from no apparent disturbance to severe obstruction.

The present study describes a unique case of amyloidosis of Waldeyer's ring and electron microscopic observations of large amyloid deposits and the cells surrounding them in palatine tonsils.

CASE REPORT

A 35-year-old male patient, born in Morocco, was admitted to our department in February 1977. Six years prior to the present admission he was treated for acute maxillary sinusitis. Three months before admission he had an attack of acute tonsillitis, which was treated successfully by ampicillin. Fi e weeks later another episode of tonsillar infection occurred. He was referred to our outpatient clinic because of a mild feeling of discomfort in the throat which had persisted since the last episode of infection.

On exam nation both tonsils were found to be enlarged and firm. Several yellowish plaque were observed in their anterior surface and an additional large plaque measuring 3 0.4 m was seen over the left posterior pillar. Se eral yellowish masses occupied the base of the tongue and encroached on the epiglottis. Another mass was seen in the left vallecula. A plaque ×0.3 cm in size spread upward from the upper border of the oropharynx to the lateral wall of the nasopharynx on the left side and another small plaque was seen in the middle of the posterior wall of the nasopharynx. The patient was in good physical condition and examination revealed no other pathological findings.

Laboratory findings: peripheral blood hemoglobin 13.8–15.0 Gr%, ESR (Westergren) 10 mm in the first hour; leukocytes 5700–7700 per mm^3 with a normal differential count. Urine analysis, including microscopic examination of sediment, disclosed no abnormality. Blood levels of glucose, urea, uric acid, albumin, globulin, creatinine, bilirubin, cholesterol, electrolytes, alkaline phosphatase, SGO transaminase, prothrombin, calcium and phosphorus were all within the normal range. Creatinine clearance was 125 ml/min, serum folate 8.8 ng/ml, serum B_{12} 266 pg/ml. Serologic tests: Wassermann, Kahn, VDRL, cold agglutinins, Rose Waaler, Paul Bunnell, latex, rheumatoid factor, antinuclear factor, monotest were all negative. Anti-DNA level was 12.1%. LE cells were not found. Measurement of blood immunoglobulins showed IgA 168 mg%, IgM 67 mg% and IgG 1830 mg%.

In vitro stimulation of peripheral blood lymphocytes by lectin mitogens (phytohemagglutinin, Concanavalin-A, pokeweed mitogen) was normal.

Tonsillectomy and biopsy of the nasopharyngeal and hypopharyngeal tissue masses were done. All the studies revealed amyloidosis as determined by Congo Red staining. Biopsies of rectal and buccal mucosae did not show any signs of amyloid deposits.

METHODS

Different regions from the excised right tonsil were fixed in 2.5% glutaraldehyde in cacodylate buffer, pH 7.4, for 2 hours and postfixed in 1% osmium tetroxide for one hour. Following dehydration in a graded ethanol series and propylene oxide, they were embedded in Epon 81 (Luft 1961). Semithin sections were stained with toluidine blue; ultrathin sections were stained with uranyl acetate and lead citrate.

Acta Otolaryngol 89 562–569 1980

AMYLOIDOSIS OF WALDEYER S RING

A Clinical and Ultrastructural Report

M Beiser [1] G Messer [2] J Samuel [1] B Gross [2] and E Shanon [1]

From the Department of Otolaryngology and Laboratory of Elect on Microscop Ichilov Medical Center Tel Aviv and S ckler School of Medicine University of Tel A h Israel

(Received May 29 1979)

Abstract Amyloidosis of the tonsil is a rare condition and amyloidosis of Waldeyer s ring has not been previously reported The present study describes a case of amyloidosis of the tonsil nasopharynx and base of tongue in a 35-year-old patient in otherwise good health and without clinical symptoms. The possibility of systemic amyloidosis was excluded by clinical examination and biopsy of rectal and buccal mucosae Electron microscopy showed the presence of typical amyloid fibrils Large masses of the fibrils were closely surrounded by cells which appeared to be phagocytic The ultrastructure of these cells is described This case is considered to be a unique type of organlimited amyloidosis of Waldeyer s ring

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Over the years numerous classifications of amyloidosis have been proposed based on diverse criteria Early classification (King 1948) was based on the staining properties and divided amyloidosis into typical and atypical forms Other classifications were based on the pathologic mechanism (Symmers 1956) histologic distribution (Heller et al 1964) or the predominant clinical patterns (Isobe & Osserman 1974) The latest classification discussed by Glenner & Page (1976) represents a slight modification of that suggested by Symmers and further de-emphasizes the anatomic pat terns of amyloid deposition They make a distinction between amyloidosis which is re stricted to the conditions in which major rec ognizable anatomic or physiologic alteration is produced by the presence of amyloid material and localized deposits of amyloid where it is present without the production of an easily recognizable clinical abnormality

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OBSERVATIONS

By light microscopy tonsillar tissue was seen to contain numerous amyloid deposits of vary ing size and shape They appeared to impinge upon the epithelium in some instances as well as fill extensive areas of subepithelial regions Many of the large subepithelial deposits were round to oval (in shape) and were encircled by cells many of which contained numerous vacuoles (Fig. 1) some appeared to possess several nuclei.

Electron microscopy of such regions showed the deposits comprised linear non branching fibrils about 100 Å wide arranged in random array (Fig. 2) the characteristic *ultrastructural features of* amyloid fibrils (Co- ...n et al 1978 Glenner & Page 1976 Pras & ...fin 1978) The fibrils were closely apposed basal epithelial cells sometimes appearing be contiguous with them. Short cytoplasmic ...ocesses extended from the cells to the fi- ...ils (Fig. 2) Deposits also lay within deep in- ...ginations of the epithelial cells were they ...ere surrounded by long thin cytoplasmic ...rocesses (Fig. 3) The basal lamina was ab- ...ent in such regions and the epithelial cells ...ere irregular in shape with only few inter ...igitations and desmosomes between adjacent ...ells.

In the subepithelial areas the large amyloid deposits were encompassed by layers of cells and cellular processes The vacuolated cells varied in size some reaching large dimensions Fig 4) They were adjoined closely to each other *with a narrow intercellular gap in some* cases a clearly defined separation between apparently adjacent cells was not observable therefore suggesting cell fusion or multinucleate cells Their cell surface was irregular with small pinocytotic-like indentations broad undulations and numerous cell processes which extended into and around the fibrillar aggregates (Figs 4 5 6)

Inclusions containing fibrils similar to those in the extracellular space were observed in the cell periphery and cell processes (Figs 5 6) They were roundish or irregular in shape and probably represented areas of extracellular deposits which had been encompassed by the cell processes as well as grazing sections of cell invaginations enclosing extracellular fibrils The cells also contained many heterogenous vacuoles *ranging in size from* about 0.2–? μm They contained varying amounts of poorly defined fibrillar material vesicles granules dense bodies and empty regions *(Figs 6 7) or* appeared empty except for the presence of membranous and myelin-like components (Fig 8) The cells possessed nuclei with peripheral condensed chromatin and one or more nucleoli occasional dense lysosome like bodies numerous mitochondria, microfilaments *dispersed* cisternae of rough endoplasmic reticulum ribosomes and many small vesicles (Figs 7 9)

The same amyloid deposits were surrounded also by elongated cells Some of these cells and the long cell processes contained conspicuously large amounts of microfilaments frequently packed closely together In some instances there was continuity between the intracellular filaments and extracellular amyloid fibrils with no intervening plasma membrane (Fig. 10) Deterioration of cells adjacent to the amyloid deposits was often observed.

DISCUSSION

A case of amyloidosis of Waldeyer s ring is described A thorough investigation excluded the possibility of systemic amyloidosis The characteristic deposits were found in both palatine tonsils base of tongue and nasopharynx It appears that according to the criteria described by Glenner & Page (1976) this may be considered as localized organ-limited amyloidosis where the organ is the continuous lymphatic tissue comprising Waldeyer s ring It is of interest to note that this ring *of lymphatic tissue is* unique in its being exposed to the external environment. Attempts were made to clarify any potential etiological factors but no conclusions were possible





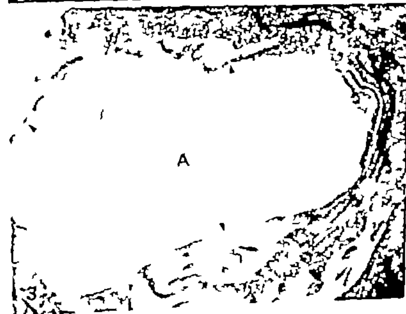
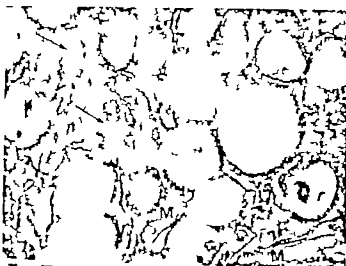


Fig 1 Semith n epon section of large amyloid deposit (*A*) surrounded by cells some of which contain numerous vacuoles. ×835

Fig 2 Electron micrograph of amyloid deposit (*A*) adjacent to epithelial cell (*E*). The amyloid fibrils are in contact with the cell periphery and cell projections (*arrow heads*) *T* tonofilaments ×15 225

Fig 3 Amyloid deposit (*A*) in invagination of epithelial cell Long thin epithelial cell processes (*arrow head*) surround the deposit. *T* tonofilament ×1 180





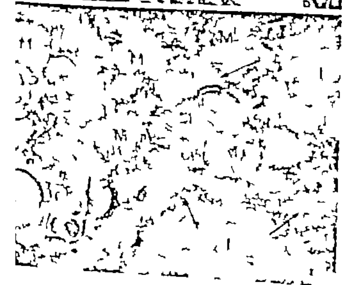


Fig. 7. Vacuoles containing varying amounts of vesicles, granular material, small dense bodies and empty regions. Note numerous profiles of RER (*arrow head*) and small vesicles (*arrow*) in the cytoplasm. *M*: mitochondria. ×13 090

Fig. 8. Clear vacuoles, some of which contain membranous and myelin-like components (*arrow*). *N*: nucleus. ×12 180

Fig. 9. Region of vacuolated cell containing numerous mitochondria (*M*) and microfilaments (*arrow*). *I*: amyloid inclusions. ×33 060

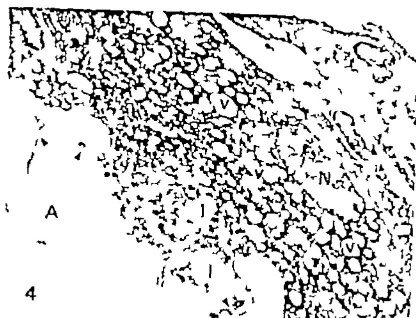


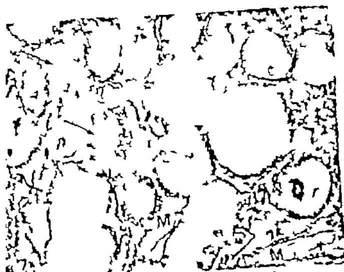




Fig 4 Part of a large cell surrounding a big amyloid deposit (*A*) Cell contains nucleus (N) numerous vacuoles (V) amyloid inclusion (*I*) and cell processes (*arrowhead*) extending into the deposit × 610

Fig 5 Higher magnification of amyloid deposit (*A*) in cell invagination and similar material in apparent cell inclusion (*I*) Plasma membranes appear to be absent in part of the inclusion and cell process Clear regions between the cytoplasm and amyloid are observed ×11 435

Fig 6 Vacuole containing fibrils (*arrow*) and sparse poorly defined fibrillar material (*arrowhead*) A dense lysosome-like body (*D*) is present *A* extracellular amyloid deposit ×20 010





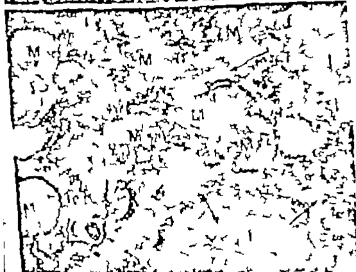
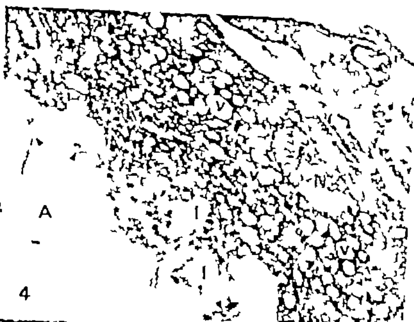


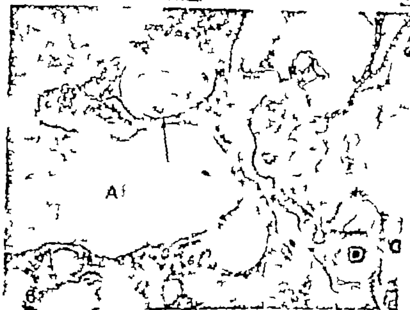
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ACKNOWLEDGEMENTS

gratefully acknowledge the advice and assistance of ... of. M. Pras, Sheba Medical Center and thank M. O ...lack and Mrs G. Buchler of the Photography Department, Municipal Governmental Medical Center, Tel-Aviv. This work was supported by the Research Committee of the Municipal Governmental Medical Center, Tel-Aviv.

ZUSAMMENFASSUNG

Amyloidose der Mandel ist ein seltener Zustand und über Amyloidosis des Waldeyer Rings wurde bisher nicht berichtet. In der vorliegenden Studie wird ein Fall von Amyloidose der Mandel, des Nasopharynx und der Zungenbasis bei einem 35jährigen gesunden Patienten ohne klinische Symptome beschrieben. Die Möglichkeit einer systemischen Amyloidosis wurde durch klinische Untersuchung und Biopsie von rektalen und buccalen Mucosae ausgeschlossen. Elektronenmikroskopie zeigte das Vorhandensein typischer amyloider Fibrillen. Große Mengen der Fibrillen waren von Zellen, die phagocytisch erschienen, dicht umschlossen. Die Ultrastruktur dieser Zellen wird beschrieben. Bei diesem Fall handelt es sich um einen eigenartigen Typ organbeschränkter Amyloidose des Waldeyer Rings.

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E. Shanon, M.D.
Department of Otolaryngology
Municipal Governmental Medical Center
Ichilov Hospital
6 Weizman Street
Tel-Aviv
Israel



Fig 10 Cell process adjacent to amyloid deposit (*A*) The cytoplasm contains numerous filaments (*arrow heads*), some in bundles (*arrow*) Filaments and part of cytoplasm appear continuous with the extracellular amyloid fibrils. ×14 790

Electron microscopic studies showed the presence of extensive typical extracellular amyloid deposits with close relationship to adjacent cells similar to findings described for other amyloidotic tissues (Cohen 1967 Glenner & Page 1976) Moreover cells surrounding some of the deposits may be considered phagocytes by their ultrastructural characteristics The cellular inclusions containing fibrils likely represent endocytic vacuoles while the heterogenous ones represent stages of digestive vacuoles active in the breakdown of the fibrils The vacuoles observed here resemble greatly those found in experimental ultrastructural studies of phagocytic uptake and processing of amyloid fibrils by macrophages by Shirahama & Cohen (1971) and leukocytes by Zucker Franklin (1970) Other experimental studies on amyloid resorption have indicated uptake of amyloid by multinucleated foreign body type giant cells as well as macrophages and leukocytes (Laufer et al 1976 Polliack et al 1970 Richter 1954 Spors 1971 Williams 1967 Wright et al 1972)

Ultrastructural evidence for resorption of amyloid by phagocytosis in human amyloidosis has been reported in familial amyloid nephropathy by Weiss & Page (1973) Multinucleated giant cells were implicated in the phagocytic activity They contained fibril-containing vacuoles similar in composition to those described here Glenner & Page (1976) have noted that the mechanism of amyloid resorption in man is unknown and suggested that macrophage activity is likely to be implicated The findings presented here support that suggestion

Perusal of the available literature disclosed reports on three cases of amyloid deposits in the tonsils The first case (Multschler 1933) was a woman aged 35 with an amyloid tumor in the lower pole of the right tonsil The second (Chiari 1937) involved an almost complete amyloid degeneration of the left tonsil in a woman aged 58 years There is only one case report on a bilateral tonsillar involvement (Eriksen 1970) Massive amyloid deposits caused an almost complete obstruction of the oropharynx According to Glenner's classification the first case (Mutschler 1933) appears to be one of a localized deposition of amyloid On the other hand the other two patients as well as the one observed by us represent an organ-limited amyloidosis Our case is unique in that it involves all elements of Waldeyer's ring This specific topography suggested an immune local response

Table I *Spontaneous cytotoxicity of tonsil and blood leucocytes of children (A) and adults (B) with chronic tonsillitis and of children with tonsillar hypertrophy (C)*

I spontaneous cytotoxicity of tonsil leucocytes
II=spontaneous cytotoxicity of blood leucocytes
III=spontaneous cytotoxicity of blood and tonsil leucocyte mixture

	% Isotope (^{51}Cr) release from target cells		
Groups	I	II	III
A	10 5±1.8 n=20	44 7± 5.8 n=8	30.5±14 7 8
B	26 6±9 8 8	55 4±11.3 8	40 8±10.5 n=8
C	12 6±1 7 n=20	42 3_ 8.7 n=9	46 6± 8 7 9

obtained by the method of Passaleva et al (1974)

Erythrocytes of chicks not more than 4 months old were used as target cells. Erythrocytes obtained in sterile conditions were treated with ^{51}Cr for an hour at 37°C The basic experiment was conducted as described earlier (Gjulling & Melnikov 1977)

The immunofluorescent method (Kubica, 1967) was used to elucidate the nature of cells accumulating in dog s tonsils during inflammation Sections of experimental and control tonsils approximately 10 μ thick were prepared on the 3rd and 8th days after inoculation The sections were treated with immune rabbit serum obtained by means of repeated immunization of rabbits with dog thymocyte membranes After serum depletion by bone marrow cells its lymphocytotoxic titer was 1 20 (according to 50% destruction of live fresh thymocytes)

Then sections were treated with ass serum against rabbit globulins labelled with FITC

The results were evaluated by Student s *t* test

RESULTS

The data presented here indicate that blood cells of patients with tonsillitis destroy actively the chicken erythrocytes with a weak metabolic activity (Table I)

Addition of autologous tonsil cells of children with tonsillar hypertrophy to blood leucocytes increases cytolysis of heteroerythrocytes in contrast to addition of tonsils cells of patients with chronic tonsillitis which produces an inhibiting effect. Thus instead of supposed additive effect of tonsil and blood cell cytolytic activity the tonsil cells of patients with chronic tonsillitis suppress the killer function of blood leucocytes

Table II shows that spontaneous cytotoxicity of intact animal tonsil cells is comparatively not high but it increases significantly in acute and chronic tonsillitis.

Cytotoxicity of dog blood cells does not differ from that of humans it does not change on the 3rd day and decreases slightly on the 8th day after induction of infection in tonsils

Destruction of heteroerythrocytes attains the level of an additive effect of separate tonsil and blood cell populations after addition of intact animal tonsil cells to autologous blood leucocytes But cytotoxicity of mixed popula-

Table II *Spontaneous cytotoxicity of blood and tonsil leucocytes of normal dogs (A) animals with experimental tonsillitis on the 3rd (B) 8th (C) day after infection induction and 1 month after infectious challenge for 3 times (D)*

I=spontaneous cytotoxicity of tonsil leucocytes
II spontaneous cytotoxicity of blood leucocytes
III=spontaneous cytotoxicity of blood and tonsil leucocyte mixture

	% Isotope (^{51}Cr) release from target cells		
Groups	I	II	III
A	8.2± 4 0 n=5	53 0_ 3 0 n=5	59.6± 7.29 n=5
B	90.6±13 6 n=3	51 0± 5.5 n=3	59.6± 7.9 n=3
C	15.6± 3 0 n=8	40 0±10.1 n=3	26 6±10 7 n=3
D	21.3± 4 4 n=4	53.6± 6 1 n=4	43 0± 4 4 n=4

Acta Otolaryngol 89 570–572 1980

TONSILLAR SUPPRESSORS OF KILLER CELLS IN TONSILLITIS

E V Gjulling and O F Melnikov

From the Department of Pathophysiology the Institute of Otolaryngology Kiev USSR

(Received April 23 1979)

Abstract The authors studied the effect of tonsil cells obtained from intact and inflamed tonsils on spontaneous cytotoxicity of autologous blood leucocytes. It has been shown that addition of tonsil cells of patients with chronic tonsillitis and those of animals with experimental tonsillitis to blood cells with a high cytotoxic potential did not augment cytolysis of heteroerythrocytes but inhibited it. Meanwhile in the joint cultivation of tonsil cells of intact animals and children with tonsillar hypertrophy with autologous blood leucocytes the killer effect exceeded that seen in a separate use of these cells The data presented in this article indicate that non-specific suppressors capable of inhibiting spontaneous killing may appear in tonsils in the course of inflammation

Suppressor cells especially T lymphocytes were shown to be of great importance in regulation of specific and non-specific humoral and cellular immune reactions The accumulation of these cells in particular limited the intensity of immune response in its final stage (Gershon 1975 Petrov 1976)

The increase of T cell suppressor activity in infection has been observed by Corsini et al (1977) Wolf et al (1978) revealed that human T lymphocytes which proliferated under the influence of microbial antigen produced a soluble inhibitor of cell proliferation and antibody synthesis It seems logical to assume that if immunogenesis does not result in a complete elimination of antigen in proper time the appearance of suppressor cells may promote the transformation of an acute inflammatory process to a chronic one

We have studied the effect of tonsil cells obtained from intact and inflamed tonsils on spontaneous cytotoxicity of autologous blood leucocytes in order to find out whether suppressor activity of tonsil cells was changed in tonsillitis

MATERIALS AND METHODS

Tonsils and blood of 28 patients with chronic tonsillitis (8 adults aged 20 to 40 and 20 children aged 3 to 10) and those of 20 children of the same age with tonsillar hypertrophy but without clinical manifestations of tonsillitis were used

Besides tonsil and blood cells of 5 intact 4-month-old puppies and of 10 dogs of the same age with experimental tonsillitis were used in order to study the dynamics of tonsil leucocyte suppressor activity in tonsillitis

Tonsillar inflammation was induced by injection of 0 1 ml of mixture consisting of complete Freund's adjuvant (Calbiochem) and a 24-hour culture of group A β-haemolytic streptococcus The suppressor activity was studied on the 3rd and the 8th day after infective mixture inoculation as well as 1 month after infectious challenge for 3 times at 1–1 5 month intervals

Tonsil cell suspension was prepared as follows Excised tonsils were washed 4 times in Hanks solution with antibiotics (penicillin and streptomycin 100 U/ml) minced with scissors and passed through a nylon filter into neutral glass bottles The cell suspension was washed twice with Hanks solution at 4°C centrifuged (150 g 3–4 min) and diluted with 199 medium to a final concentration of 5×10^7 cells/ml (Gjulling & Melnikov 1977) Blood leucocytes were

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tion of these cells is lower than a supposed additive effect when cells from inflamed tonsils are used

Thus the results of this study show that cells capable of suppressing the non specific killing appear in tonsils during inflammatory process development The addition of these cells to blood cells with a high cytotoxic potential does not augment target cell cytolysis but inhibits it Killer effect is higher in a joint cultivation of normal and hypertrophic tonsil cells with blood leucocytes than that observed in a separate cultivation of these cells

The cells with suppressor properties are already generating in inflammation focus on the 3rd day It seems possible that such cells may migrate from inflammation focus An indirect evidence of this appears to be the decrease of blood cell spontaneous cytotoxicity seen in dogs on the 8th day after infectious challenge

The immunofluorescent method shows the presence of a considerable number of fluorescent cells of lymphocyte type in tonsil sections of infected animals It seems probable that cells which suppress spontaneous cytotoxicity are T-dependent lymphocytes

Thus the study suggests that the frequent recurrences of inflammation create condition for generation of suppressor cells in inflamma tion focus these cells are supposed to inhib immune response formation and to promot the chronic course of inflammation process

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O V Gjulling MD
Research Inst of Otolaryngology
Zoologicheskaya 3
252057 Kiev 3
USSR

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Acta

OTO-LARYNGOLOGICA

VOL. 89 MAY–JUNE 1980 No 5–6

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Acta

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Conferences and Meetings

1980 July 9–16 Tenth International Congress on Acoustics to be held in Sydney *Australia.* Information 10th ICA Congress Secretariat, GPO Box 2609 Sydney NSW Australia 2001

1980, July 20–23 International Symposium on "Neuronal Mechanisms of Hearing" to be held in Prague, Czechoslovakia. Information. J Syka, Institute of Experimental Medicine, Czechoslovak Academy of Sciences, 128 08 Prague 2, U nemocnice 2, Czechoslovakia.

1980 September 2–5 IV International Symposium on Facial Nerve Surgery to be held in Los Angeles, *California, USA.* Further information address Facial Nerve Symposium, c/o Ear Research Institute, 256 South Lake Street, Los Angeles, California 90057 USA.

1980, September 21–22 The XXXI Congress of the Polish Otolaryngological Society will be *held in Poznań.* Further information. Ass Professor Antoni Pruszewicz, Clinic of Otolaryngology Academy of Medicine, 49 Przybyszewskiego St., 50 355 Poznań, Poland.

1980, September 27–28 The Research Forum, under the joint sponsorship of the Committee for Research in Otolaryngology of the American Academy of Otolaryngology and the Association for Research in Otolaryngology will be held *in* Anaheim, California. Abstract format instructions from Professor *Makoto Igarashi,* M.D., Department of Otorhinolaryngology and Communicative Sciences, Baylor College of Medicine, Houston, Texas 77030, USA.

1980 October The Ear Research Institute announces a two-week *Temporal Bone* Surgical Dissection Course. Information. Antonio De La Cruz, M.D., Director Temporal Bone Surgical Dissection Course, Ear Research Institute, 256 South Lake Street, Los Angeles, CA 90057 USA.

1980 October 3 A Meeting of the O R.S. (Oto-Rhino-Laryngological Research Society) will be held at the Royal National Throat, Nose and Ear Hospital, Gray's Inn Road, London. Information Professor P Stell, Ch.M., F R.C.S., Department of Otolaryngology Royal Liverpool Hospital, Prescot Street, Liverpool. L7 8XP England.

1981 March 22–27 Second International Conference on Cholesteatoma a Mastoid Surgery to be held in Tel Aviv Israel. Information Professor J Sadé, International Conference on Cholesteatoma and Mastoid Surg.